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ULTRAFILTRATION STUDIES ON CALCIUM AND PHOSPHORUS IN HUMAN SERUM

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There has long been interest in the status of the calcium which circulates in the extracellular fluids of the body. It is known that normally there are homeostatic mechanisms which exert precise control to maintain the *total* calcium of the serum within certain bounds (1) (9.5 and 11 mg. per 100 cc.). Of this total serum calcium a certain portion is "attached" to the proteins of the serum, and hence is supposedly non-filterable through the capillary membranes. The point of interest to the physiologist is that concentration of calcium which bathes the tissue cells—i.e., the calcium concentration of the extracellular fluids. McLean and Hastings, by their ingenious bioassay technique using the frog's heart, determined that in the normal individual approximately one-half the total serum calcium is diffusible and that to all intents and purposes this is completely in ionic form (4.25 to 5.25 mg. per 100 cc.) (1). It seems reasonable to accept the dictum that only calcium ions are active in influencing nerve and muscle excitability, but there may be doubt whether diffusible but non-ionized calcium such as calcium citrate and calcium "attached" to protein might not, under certain circumstances, be available to cells for metabolic purposes such as the calcification of bone and cartilage matrix.†

Despite the likelihood of some variations in the protein concentration of extracellular fluid, it seemed desirable to obtain further knowledge of how calcium is "carried" in the *serum* under a variety of conditions, simulating pathological states in man. Some of these disease states include multiple myeloma and sarcoidosis, where serum calcium may be high or normal, independent of the presence of elevated total serum protein concentration or abnormal ratio of albumen to globulin; renal insufficiency where the serum phosphorus is nearly always elevated and the calcium may be either low or normal; in hyperparathyroidism where the calcium is elevated and the phosphorus low (except with renal insufficiency) (2, 3).

METHODS

Serum calcium determinations were done by Clark and Collip modification of the Tisdall method (4). Phosphorus analyses were done by the Umbreit modification (5) of the Fiske-

* The work described in this paper was carried out under a contract between The Johns Hopkins University and the Atomic Energy Commission.

† It seems probable that under varying dynamic conditions considerable amounts of protein may pass capillary membranes.

Subbarow method (6). Magnesium was determined as magnesium phosphate by the method of Denis (7), modified in that the phosphate was determined by Umbreit's method (5). Serum magnesiums were run in duplicate and the results were accepted only when there was agreement within 0.3 mg. per 100 cc. It is believed that all of the calcium present in the sera and ultrafiltrates is recovered by the method used (4) and that magnesium present does not affect the results for the following reasons: when magnesium is added to the serum in known quantities, the total calcium determination on a given quantity of serum remains the same, and the magnesium may be quantitatively recovered from the supernatant after quantitative calcium precipitation. When calcium is added in known amounts to serum with added magnesium, the former may be quantitatively recovered. Total serum nitrogen was determined by the macro-Kjeldahl procedure of Campbell and Hanna (8). An arbitrary figure of 30 mg. per 100 cc., representing non-protein nitrogen, was used in the calculation of total serum protein concentration from total serum nitrogen concentration. Serum sodium and potassium were determined by flame photometry (9, 10a, 10b). Serum chloride was determined by the method of Schales and Schales (11). The Beckman pH meter (model G) was used for all pH determinations. The pH readings were made at room temperature (25–28°C) and a temperature correction factor was not applied.

TABLE I

Effect of Ultrafiltration Pressure on Ultrafilterable Calcium
Serum Calcium Concentration—9.0 mg.%

PRESSURE MM. Hg	FILTRATE Ca MG. %	% FILTERABLE Ca	FILTRATE pH
100	6.5	72	7.52
150	6.2	69	7.43
200	6.3	70	7.45

The method chosen for obtaining an ultrafiltrate of serum was that devised by Lavietes (12), because with this technique the ultrafiltration is carried out anaerobically and pH changes due to loss of CO_2 are prevented. This method is described in some detail in the appendix.

RESULTS

In preliminary experiments it was found that, in order to obtain quantities of ultrafiltrate adequate for analysis within reasonable periods of time, a pressure gradient of at least 200 mm. of mercury is required. Elevation of the pressure gradient to more than 240 mm. mercury results in 20 to 30% breakage of the cellophane membrane. All of the experiments herein reported (unless otherwise stated) have been carried out at 200 mm. Hg pressure. Lowering of the pressure gradient to the physiological range of capillary pressure (15 to 25 mm. Hg) resulted in recovery of an insignificant quantity of ultrafiltrate over periods of 48 hours. The method is thus not a true reflection of physiological conditions in terms of filtration pressure. However, pressure gradients between 100 mm. Hg and 200 mm. Hg resulted in no significant change in the concentration of calcium in the ultrafiltrate (Table I).

The expression "% filterable Ca" or "% filterable P" is used throughout the tables and text to denote the ratio between the calcium or phosphorus concentrations in the ultrafiltrate of the serum and the original serum. The calcium concentration in the ultrafiltrate (omitting a small correction factor for the Donnan equilibrium effect) is considered to equal the calcium concentration in the *water* of the serum. The "% filterable Ca" denotes, therefore, the portion of calcium unattached to protein in the original serum, while the difference between this value and 100% denotes the portion of calcium "bound" to protein. In Table II it may be seen that when samples of a single pool of serum are ultrafiltered for 16, 24 and 48 hours, the calcium concentrations of the ultrafiltrates do not differ significantly. It is concluded from this that, as the volume of ultrafiltrate and the protein concentration of the residue increase with longer duration of ultrafiltration, the calcium-protein relationship is unchanged during the ultrafiltration procedure. Because of this it is necessary to allow each ultrafiltration to proceed only long enough to secure adequate

TABLE II

Effect of Duration of Ultrafiltration on Ultrafilterable Calcium

HOURS FILTERED	POOL #4 SERUM Ca—9.7 MG.%			POOL #14 SERUM Ca—9.4 MG.%		
	Filtrate pH	Filtrate Ca mg.%	% filterable Ca	Filtrate pH	Filtrate Ca mg. %	% filt. Ca
16	7.48	7.4	76	7.46	6.3	67
24	7.58	7.0	73	7.50	6.3	67
48	7.50	7.2	74	7.48	6.4	68

amounts of ultrafiltrate for analysis. This constitutes an advantage of this method over such methods as compensation dialysis and vividialysis in which care must be taken to allow the system to come to equilibrium. However, in all subsequent experiments 24 hours were allowed for each ultrafiltration.

A check on the accuracy of method is that total calcium of the ultrafiltrate plus the total calcium of the residue, invariably add up to the quantity of calcium known to be present in the original sample—thus denoting 100% recovery (plus or minus 3% which is within limits of error in measurement of residue and filtrate, and includes the ±0.2 mg. per 100 cc. error of the method for calcium determination). (4) This recovery is also evidence that no significant amount of calcium is lost by adsorption on the cellophane membrane, and such recovery has been invariably present in all earlier experiments to be described. In later experiments duplicate determinations on the filtrate and the control serum have checked within 3 per cent.

It is believed that no significant quantity of protein passes the membrane during ultrafiltration. A method of micro-digestion and Nesslerization, without

addition of protein precipitants, was used in determining the total nitrogen of the ultrafiltrates. Nitrogen determinations were carried out on 11 samples of ultrafiltrate from normal serum (non-protein nitrogen of serum about 30 mg. per 100 cc.), and the results showed nitrogen content varying from 35 to 47 mg. per 100 cc. The biuret test on numerous ultrafiltrates was negative.

All sera used were of human origin. The analyses were carried out on fresh sera or sera frozen immediately and later thawed, in order to avoid any possible changes in composition that might result from standing or bacterial contamination. It was found that such freezing procedures did not alter either the total calcium or the ultrafilterable calcium as compared with the same sera

TABLE III

Effect of Freezing on Ultrafilterable Calcium

Serum Ca = 10.6 mg.%

FILTRATE pH	FILTRATE Ca MG.%	% FILTERABLE Ca	
7.62	6.9	65	Filtered fresh
7.72	6.9	65	Frozen for 20 days, thawed and saturated with 5% CO_2 and 95% O_2
7.68	6.8	64	Frozen for 34 days, thawed and saturated with 5% CO_2 and 95% O_2

TABLE IV

Effect of Temperature on Ultrafilterable Calcium

POOL	SERUM Ca MG.%	26°C.			38°C.		
		Filtrate pH	Filtrate Ca mg.%	% filt. Ca	Filtrate pH	Filtrate Ca mg.%	% filt. Ca
2	10.5	7.61	6.8	65	7.51	6.6	63
14	9.4	7.50	6.3	67	7.40	6.4	68
43	9.6	7.35	6.8	71	7.30	6.5	68

when tested in the freshly drawn state, provided the pH was adjusted after thawing (Table III).

There was likewise no significant difference between results of ultrafiltration when the procedure was carried out at 26° C. and 38° C. (Table IV).

Effect of pH changes on the ultrafilterable calcium fraction

Pooled serum was tested at different pH—changes in pH having been produced by additions of small amounts of saturated solution of sodium carbonate or dilute hydrochloric acid. Tests were run from pH 9.9 to 5.1. It was found that alkalinization of the serum reduced the calcium concentration of the ultrafiltrate; acidification increased it. At pH 9.9 the calcium concentration of the ultrafiltrate is approximately 30 per cent of the calcium concentration of the unfiltered serum; at pH 5.1 the calcium concentration in the ultrafiltrate

is 97 per cent of that of the unfiltered serum (Table V). These findings are in agreement with Smith (13).

When frozen pooled serum is thawed, it is found to have a pH in the region of 8 or slightly higher. At this pH the calcium concentration of the ultrafiltrate is 1 to 2 mg. per 100 cc. lower than the calcium concentration of the ultrafiltrate when the same serum has been acidified by saturation with 5% CO_2 in 95% O_2.* (Table VI). A narrower range of pH values was obtained by adding very small amounts of N/5 HCl to aliquots of a pool of serum which had been

TABLE V

The Effect of pH on Ultrafilterable Calcium and Phosphorus

NO.	pH ADJUSTED WITH	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	SERUM P MG.%	FILTRATE P MG.%	% FILT. P
2B	Sat. sol. Na_2CO_3	9.90	10.2	3.3	32	3.9	2.5	64
2K	Sat. sol. Na_2CO_3	9.88	10.2	3.0	29	3.9	2.7	69
2D	Loss of CO_2	8.28	10.5	5.2	50	4.1	4.0	98
2F	Sat. with 5% CO_2–95% O_2	7.57	10.5	7.2	69	4.1	4.4	107
2G	Dilute HCl	5.69	10.5	9.2	88	3.8	3.6	95
2C	Dilute HCl	5.12	10.5	10.2	97	3.9	4.1	105

TABLE VI

Effect of Variation in CO_2 Content of Serum on Ultrafilterable Ca and P

POOL NO.	SERUM Ca MG.%	SERUM P MG.%	SERUM T.P. GM.%	LOSS OF CO_2 WITH FREEZING AND THAWING						SATURATION WITH 5% CO_2 – 95% O_2					
				Filtrate pH	Filtrate Ca mg.%	% filt. Ca	pK Ca Prot.	Filtrate P mg.%	% filt. P	Filtrate pH	Filtrate Ca mg.%	% filt. Ca	pK Ca Prot.	Filtrate P mg.%	% filt. P
1	10.4	4.1	6.5	8.18	5.5	53	1.99	4.4	107	7.65	7.2	69	1.69	4.3	105
2	10.5	4.1	6.1	8.28	5.2	50	2.07	4.0	98	7.57	7.2	69	1.72	4.4	107
13	9.5	4.2	7.0	8.23	5.5	58	1.90	4.2	100	7.47	6.8	72	1.64	4.0	95

frozen and then thawed. A significant decrease in percentage of ultrafilterable calcium with increase in pH from 7.26 to 7.87 may be seen in Table VII.

It is, therefore, highly important to readjust the serum before ultrafiltration to a known and fixed pH in order to obtain comparable values. In the literature may be found data which affirm this statement (13, 14, 15, 16); other investigators have denied that pH changes in this range have any effect on the quantity of ultrafilterable calcium (17, 18, 19). In all our experiments,

* Saturation of sera with 5% CO^2 in 95% oxygen is accomplished by using a 125 ml. Erlenmeyer flask with a 2-holed rubber stopper, one opening for the gas intake and the other acting as gas outlet. The flask containing the sera is rotated to saturate it with 5% CO_2 in 95% oxygen, and after three minutes the sera is immediately introduced into the ultrafiltration chamber.

unless otherwise stated, the pH has been adjusted by saturation with 5% CO_2 in 95% oxygen; but the resulting pH is variable, from 7.2 to 7.7. The pH values are fairly consistent for any given pool of serum, but vary from pool to pool.

In Table VIII may be seen the degree of ultrafilterability of various electrolytes in three different serum pools. The percentages of ultrafilterable ions may be recalculated on the basis of the *water* content of their respective serum pools by employing the factor 0.75 for the specific volume of serum proteins and allowing 1% of the total volume of both sera and ultrafiltrates for solids other than proteins (20). When this is done, it may be seen that in three pools

TABLE VII

The Effect of Small pH Changes on Ultrafilterable Calcium

T.P. = 6.6 gm.%

NO.	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILTERABLE Ca	pK Ca PROT.
19A	7.26	9.2	6.9	75	1.59
19G	7.36	8.9	6.7	75	1.58
19B	7.47	8.9	6.7	75	1.58
19C	7.50	9.0	6.6	73	1.63
19D	7.63	8.9	6.3	71	1.68
19E	7.71	9.1	6.0	66	1.78
19F	7.87	8.9	5.7	64	1.82

TABLE VIII

POOL	FILTRATE pH	SERUM Ca MG.%	FILT. Ca MG.%	% FILT. Ca	CORR. %*	SERUM P MG.%	FILT. P MG.%	% FILT. P	CORR. %*	SERUM Cl meq/L	FILT. Cl meq/L	% FILT. Cl	CORR. %*	SERUM Na meq/L	FILT. Na meq/L	% FILT. Na	CORR. %*	SERUM K meq/L	FILT. K meq/L	% FILT. K	CORR. %*	T.P. GM. %	pK Ca PROT.
40	7.42	9.0	6.3	70	66	5.3	5.4	102	97	101.0	111	110	105	143.5	147.5	103	97	4.7	4.9	104	98	7.2	1.65
42	7.52	10.3	6.5	63	60	5.1	5.2	102	97	105.5	116	110	105	151.0	155.0	103	98	4.4	4.8	109	104	6.7	1.83
43	7.42	9.6	6.8	71	67	4.0	4.6	115	109	104.5	114	109	104	145.0	147.5	102	97	4.5	4.8	107	102	6.8	1.68

* Calculated on the basis of the *water* content of the serum and ultrafiltrate.

the percentages of ultrafilterable sodium varied from 97 to 98% and that of potassium from 98 to 104%. These are slightly higher than the expected values of 95% calculated on the basis of the Donnan equilibrium effect on wholly filterable cations, and it may be assumed, therefore, that sodium and potassium are wholly filterable. This was demonstrated by Folk et al (21) who compared the distribution of these electrolytes between the serum and certain extracellular fluids in man. In Table VIII it may be seen that the percentage of ultrafilterable chloride varied from 103 to 105% as would be expected for a wholly filterable anion (20).

When the percentages of ultrafilterable phosphorus from six different pools of serum subjected to ultrafiltration between pH 7.42 and 7.65 (Tables VI and

VIII) are recalculated on the basis of the *water* content of the serum, they are found to vary from 90 to 109% and average 99%. This scattering of values is considerably beyond the limits of experimental error. If phosphorus were a wholly filterable anion in physiological pH range, a value of 105% for the ultrafilterable phosphorus would be obtained. Whenever this percentage *corrected on the basis of water content of the serum* is significantly lower than 105%, or the uncorrected percentage is less than 111%, one must conclude that the inorganic phosphorus in that instance is not wholly filterable. From our results it would appear that a small and inconstant proportion of the inorganic phosphorus of serum is not ultrafilterable, perhaps due to a complex involving protein. Such a situation is undoubtedly present at higher pH levels (Table V). In the literature the work of several investigators, when judged by the above criterion, appears to demonstrate that the inorganic phosphorus of human serum is not wholly ultrafilterable (22, 23, 24). In normal dog serum, which has a higher inorganic phosphorus concentration than human serum, there is more evidence for the existence of a small amount of non-filterable phosphorus at physiological pH as demonstrated both by vividiffusion (25) and by ultrafiltration techniques (13, 26). When Grollman poisoned dogs with parathyroid extract, thus raising both serum calcium and phosphorus, he found reduced ultrafiltration of the serum phosphorus (27).

pK values expressing the theoretical dissociation constants for calcium proteinate in seven different pools of serum within the pH range of 7.42 to 7.65 have been calculated according to the method of McLean and Hastings (20) and may be seen in Tables VI, VII and VIII to lie within the range of 1.58 to 1.83. The pK values for calcium proteinate within a single pool of serum increase with rise in pH (Table VII). This method of calculation (20)* may not be strictly valid for an ultrafiltration system where conditions are dynamic (in contrast to the static conditions of the frog heart method (1) for determining ionic calcium). However, we believe its use is justifiable on the basis of the data presented in Table II which show that the concentration of calcium in the ultrafiltrate is not constantly changing, at least within the period from 16 to 48 hours after ultrafiltration is begun. Assuming then the ultrafiltrate calcium concentration to be a constant, the other factors in the calculation

*pK equals the negative log of the dissociation constant K for calcium proteinate.

$$K = \frac{(Ca^{++})(Prot^{=})}{(Ca\ Prot)}$$

(Ca^{++}) as used here = the ultrafilterable Ca concentration expressed in moles per kilo of water.

($Prot^{=}$) = dissociated base-combining groups of protein expressed as milliequivalents divided by two. The factor used was 0.243 which assumes an A/G ratio of 1.8 and a pH of 7.35. (Ca Prot) denotes the Ca bound to protein and = (Total Ca) minus (Ca^{++}) when both are expressed in moles per kilo of water.

are also constants and the derivation is permissible. It should be emphasized that the proteins referred to in this derivation are the normal serum proteins with an albumen-globulin ratio of 1.8. Therefore, the pK values may vary from pool to pool with variations in this ratio.†

TABLE IX

The Effect of Increased Serum Calcium on Ultrafilterable Calcium and Phosphorus

NO.	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	FILTRATE VOL. CC.	RESIDUE Ca MG.%	RESIDUE VOL. CC.	SRERM P MG.%	FILTRATE P MG.%	% FILT. P	SERUM PROT. GM.%	MG. Ca BOUND BY 1 GM. PROT.	pK Ca PROT.
2F	7.57	10.5	7.2	69	4.8	15.6	3.2	4.1	4.4	107	6.1	0.59	1.76
2M	7.59	17.9	12.3	69	5.1	26.9	3.4	3.7	3.9	105	5.8	1.1	1.79
2J	7.59	19.6	12.6	64	5.4	30.9	3.5	3.7	3.7	100	5.7	1.3	1.88
2L	7.65	25.4	17.0	67	5.6	38.2	3.5	3.6	3.1	86	5.6	1.6*	1.84*

* These figures are unreliable since some of the calcium calculated to be bound by protein may actually be bound in the hypothetical phosphate complex.

To 15 cc. aliquots of thawed pooled serum were added varying amounts of an approximately 0.4% calcium chloride solution. One half of each sample was subjected to anaerobic ultrafiltration, the other half analyzed as control. All samples were saturated with 5% CO_2 in 95% oxygen after addition of the $CaCl_2$ prior to ultrafiltration. The decrease in total protein concentration from sample to sample is a result of dilution by the added $CaCl_2$ solution.

† Sample calculation:

2F: 4.8 cc. filtrate × 7.2 mg.% Ca = 0.35 mg. Ca in filtrate
3.2 cc. residue × 15.6 mg.% Ca = 0.50 mg. Ca in residue
8.0 cc. serum × 10.5 mg.% Ca = 0.84 mg. Ca in serum

Residue plus filtrate Ca = 0.85 mg. Ca or 101% recovery.

Using the formula: Water in gm./100 cc. serum = 99.0 minus 0.75 × protein concentration in gm.% (20) the water content of 8.0 cc. serum #2F is $\frac{8.0 \times (99.0 - 0.75\ (6.1))}{100}$ = 7.6 cc. The volume occupied by protein (and other solids) in this sample is, therefore, 0.4 cc. At the end of filtration all of the protein is in the residue. Therefore, the residue water = 3.2 − 0.4 or 2.8 cc. According to the Donnan equilibrium, the calcium concentration in the residue water should be approximately 1.05 times the filtrate calcium concentration (20) or 1.05 × 7.2 = 7.6 mg.%. Total calcium in the residue water would then equal 2.8 cc. × 7.6 mg.% = 0.21 mg. However, by analysis there is 0.50 mg. Ca in the residue. The difference or (0.50 − 0.21) = 0.29 mg. Ca represents the amount bound by protein. Since the serum protein concentration is 6.1 gm.%, there is $\frac{8.0 \times 6.1}{100}$ or 0.49 gm. protein in the residue at the end of filtration. If 0.49 gm. protein binds 0.29 mg. Ca, then 1 gm. protein binds 0.59 mg. Ca at this serum Ca concentration.

Effect of addition of calcium to serum on the concentration of calcium and phosphorus in the ultrafiltrate

When calcium is added to serum *in vitro* so that the total calcium ranges between 10.5 and 25.4 mg. (Table IX), the percentage of ultrafilterable calcium remains essentially constant. Furthermore, the calculated pK values

† The proteins referred to are normal serum proteins with normal A/G ratios; but since these are a mixture of many proteins, the K is not truly a physicochemical "constant."

remain essentially constant throughout this range of serum calcium concentration. This fact suggests that mass action effect is operating under these circumstances. Using the method of calculation expressed below Table IX, it can be seen that with rising total calcium the quantity of calcium proteinate increases, in agreement with Smith and Sternberger (28). The inorganic phosphorus concentration in the ultrafiltrate is approximately 105% of its concentration in the serum *until* the calcium concentration of the serum reaches a level between 19.6 and 25.4 mg. per 100 cc. when there appears a sharp fall in the phosphorus concentration of the ultrafiltrate.

Another experiment along similar lines was so designed that the protein concentration would remain constant despite a wide range of calcium concentration. In Table X it may be seen that when the serum calcium is raised to

TABLE X

The Effect of Increased Serum Ca on Ultrafilterable Ca and P

SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	SERUM P MG.%	FILTRATE P MG.%	% FILT. P	FILTRATE pH	pK Ca PROT.
7.9	5.2	66	3.6	3.8	106	7.52	1.75
9.7	6.5	67	3.9	3.7	95	7.49	1.73
11.1	7.5	68	3.7	3.9	105	7.49	1.72
12.3	8.9	72	3.7	3.7	100	7.52	1.62
14.3	9.9	69	3.7	3.7	100	7.48	1.69
17.4	12.2	70	3.7	3.7	100	7.48	1.67
20.4	13.1	64	3.8	2.6	68	7.44	1.79*

* Unreliable since a calcium phosphate complex may be present.

0.4% $CaCl_2$ was added to pooled serum in increasing amounts, each sample of serum being 21 cc.; all the samples prior to ultrafiltration were diluted to 25 cc. by the addition of distilled water. The protein concentration of each sample was thus identical.

some point between 17.4 and 20.4 mg.%, there is a decrease in the percentage of ultrafilterable phosphorus. But in this experiment, in contrast to the former, the percent of ultrafilterable calcium falls, perhaps due to the formation of a non-filterable complex involving calcium, phosphate and probably protein.

Another experiment was carried out to define more exactly that concentration of serum calcium at which ultrafilterable phosphorus falls below its normal level. To pooled serum with normal phosphorus concentration, $CaCl_2$ was added so that the calcium concentraton in four of the specimens was between 17 and 21 mg. per 100 cc. (Table XI).

It was again found that with normal concentration of serum phosphorus, the ultrafilterable phosphorus fell when calcium concentration was greater than 19 mg.%. In this experiment ultrafilterable calcium fell sharply coincidently with the fall in ultrafilterable phosphorus. However, no clouding appeared and no sediment was obtained on centrifugation of the original sample. In this

experiment and those described hereafter, duplicate calcium analyses were done on the serum and the filtrate, and the residues were not analyzed.

Effect of altering serum phosphorus concentration

When inorganic phosphorus was added to samples of serum so that concentrations from 4.1 to 34.1 mg. per 100 cc. were established (Table XII), nearly all the phosphorus proved to be ultrafilterable and the percentage of ultrafil-

TABLE XI

The Effect of Increased Serum Ca on Ultrafilterable Ca and P

Total Protein = 6.2 gm.%

FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	SERUM P MG.%	FILTERABLE P MG.%	% FILT. P	pK Ca PROT.
7.40	14.0	9.9	71	3.9	3.9	100	1.72
7.40	17.3	12.7	73	4.0	3.9	98	1.66
7.40	18.4	12.8	70	4.0	4.0	100	1.74
7.41	19.4	13.5	70	4.0	4.0	100	1.74
7.46	20.3	11.0	54	3.9	2.9	74	2.02*

* Inaccurate due to probable calcium phosphate complex formation.

TABLE XII

Effect of Increased Serum Phosphorus on Ultrafilterable Calcium and Phosphorus

Total Protein = 6.5 gm.%

NO.	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% OF FILTERABLE Ca	SERUM P MG.%	FILTRATE P MG.%	% OF FILTERABLE P	pK Ca PROT.
14J	7.45	8.6	6.6	77	4.1	4.4	107	1.55
14K	7.40	8.9	6.1	69	12.4	13.1	106	1.74
14L	7.30	8.5	6.4	75	20.4	22.0	108	1.60
14M	7.32	8.3	6.2	75	29.4	31.0	105	1.60
14N	7.30	7.6	5.1	67	34.1	36.9	108	1.76

To 10 cc. aliquots of pooled serum were added increasing amounts of a buffer solution containing Na_2HPO_4 and KH_2PO_4 (pH 6), plus sufficient 0.9% saline to bring the samples to 11 cc. volume. No precipitate was visible on centrifugation of the samples after addition of the phosphate solution and prior to ultrafiltration.

terable calcium did not fall significantly. Similar results in regard to ultrafilterable phosphorus were obtained after phosphate injections in the dog by Smith et al (26). Furthermore, McLean and Hastings found that additions of buffered phosphate solution in concentrations up to 10 mmol per liter had no effect on the ionized calcium (20).

Ultrafiltrations of the samples of serum reported in the preceding experiments were carried out at varying lengths of time up to a week after addition of the excess calcium or phosphate ions. Since preliminary experiments indi-

cated that there might be a delayed equilibrium in the formation of a calcium-phosphate "complex",* as there is in precipitation of calcium phosphate from supersaturated solutions (29), the following ultrafiltrations were carried out within 24 hours of the addition of excess ions to the serum.

The experiment shown in Table XIII was performed to determine at what level of serum phosphorus a calcium-phosphate complex might be formed at physiological pH in the presence of a *normal* serum calcium concentration.

TABLE XIII

The Effect of Increased Serum P on Ultrafilterable Ca and P

Total Protein = 6.2 gm.%

NO.	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	SERUM P MG.%	FILTRATE P MG.%	% FILT. P
32A	7.36	10.3	6.3	61	10.1	10.6	105
32B	7.35	10.9	6.8	62	14.0	14.5	104
32C	7.36	10.9	5.9	54	16.9	17.2	102
32D	7.30	10.0	4.9	49	23.7	23.6	100

To 20 cc. aliquots of a single pool of serum were added 0.3 cc. of a 0.4% $CaCl_2$ solution and increasing amounts of an isotonic phosphate buffer solution (1.55 gm. $NaH_2PO_4 \cdot H_2O$ and 5.95 gm. Na_2HPO_4 in 500 cc. water) of pH 7.35 plus sufficient normal saline to bring each sample to 22 cc. Thus each final sample was similar with respect to calcium and protein concentrations. A portion of each sample was analyzed for calcium and phosphorus and another portion was saturated with 5% CO_2–95% O_2 and subjected to immediate ultrafiltration.

TABLE XIV

The Effect of Increased Serum Ca in the Presence of a High Serum P on Ultrafilterable Ca and P

Total Protein = 6.7 gm.%

NO.	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	SERUM P MG.%	FILTRATE P MG.%	% FILT. P
30A	7.62	10.4	6.5	63	9.7	10.1	104
30B	7.52	12.7	8.2	65	9.8	9.8	100
30C	7.43	15.2	8.7	57	9.8	8.9	91
30D	7.34	17.6	10.6	60	9.8	8.3	85

As may be seen in Table XIII, the percentage of ultrafilterable calcium fell to much greater degree with rising serum phosphorus than did the percentage of ultrafilterable phosphorus.

The experiments shown in Tables XIV and XV were designed to reveal at what levels of serum calcium concentration decreased filterability of calcium and phosphorus became apparent when the serum phosphorus was elevated to a fixed value.

* The use of the term "complex" denotes merely that portions of calcium and phosphorus (as compared with normal serum) have become not ultrafilterable.

The samples were made up in a similar fashion to those of the previous experiment. In Table XIV it may be seen that with a normal serum calcium concentration and a serum phosphorus concentration of 9.7 mg.%, filtration of calcium and phosphorus are normal. However, with the serum phosphorus remaining fixed at 9.7 mg.% and rising values of serum calcium, the percentage of ultrafilterable phosphorus falls progressively. The percentage of ultrafilterable calcium does not fall markedly. In Table XV may be seen a parallel situation with the serum phosphorus concentration fixed somewhat higher. Here likewise there is a fall in the percentage of ultrafilterable phosphorus even when the serum calcium concentration is still within the normal range. However, in this experiment the percentage of ultrafilterable calcium is constantly subnormal.

One experiment was performed to determine whether the filterability of calcium and phosphorus in serum with elevated calcium and phosphorus would

TABLE XV

The Effect of Increased Serum Ca in the Presence of a High Serum P on Ultrafilterable Ca and P

Total Protein = 6.5 gm.%

NO.	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	SERUM P MG.%	FILTRATE P MG.%	% FILT. P
31E	7.42	10.4	5.5	53	15.6	16.0	103
31A	7.42	11.0	6.0	55	14.6	14.4	99
31B	7.48	13.6	4.5	33	14.6	13.5	92
31C	7.42	15.6	6.3	40	14.7	12.3	84
31D	7.38	18.3	6.4	35	14.8	10.9	74

decrease with the passage of time. $CaCl_2$ and buffered phosphate solution were added to a large pool of serum in quantity such that the final calcium concentration was 23.8 mg.% and the phosphorus concentration 5.3 mg.%. A portion of this was ultrafiltered immediately. The remainder was stored in the refrigerator at 10°C. and portions were removed at intervals for ultrafiltration. No precipitate was obtained in prolonged centrifugation, even after seven weeks. The results (Table XVI) show that although a fall in both the ultrafiltrable calcium and phosphorus denoting increased complex formation occurred in the first two weeks, no further drop occurred in the subsequent five weeks.

The effect of alterations of sodium and potassium upon the ultrafilterable calcium was studied (Table XVII).

It was found that there was no significant alteration in the percentage of ultrafilterable calcium within the approximate ranges of 130 to 260 meq. sodium per litre and 3 to 38 meq. potassium per litre.

In another experiment using a similar technique magnesium was added as

TABLE XVI

The Effect of Time on Ca-P Complex Formation

Total Protein = 6.4 gm.%

NO.	SERUM Ca MG.%	FILT. Ca MG.%	% FILT. Ca	SERUM P MG.%	FILT. P MG.%	% FILT. P	FILT. pH	REMARKS
29A	23.8	18.0	76	5.3	3.9	74	7.28	Filt. stat.
29B	23.8	13.5	57	5.3	2.7	51	7.30	Filt. 2 wks.
29C	23.8	15.6	66	5.3	3.7	70	7.38	Filt. 4 wks.
29D	23.8	14.6	61	5.3	3.8	72	7.38	Filt. 6 wks.
29E	23.8	13.9	58	5.3	4.0	75	7.42	Filt. 7 wks.

TABLE XVII

The Effect of Excess Na and K on Ultrafilterable Ca

NO.	APPROX. Na meq./L	APPROX. K meq./L	FILTRATE pH	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca
5H	Normal	Normal	7.39	9.1	6.8	75
5F	200	Normal	7.32	8.7	6.9	79
5G	260	Normal	7.40	8.9	7.1	80
21A	Normal	Normal	7.58	9.3	6.5	70
21F	Normal	38	7.55	8.7	6.0	69

To 10 cc. aliquots of a single pool of serum (5H, 5F and 5G) were added increasing amounts of a NaCl solution containing 1500 meq. sodium per litre. Water was then added to make 11 cc. samples. In 21A and 21F 14 cc. aliquots of a different pool of serum were used. To 21A 1 cc. N. saline was added. To 21F 0.8 cc. of a KCl solution containing 670 meq. potassium per litre was added.

TABLE XVIII

Effect of Increased Serum Mg on Ultrafilterable Ca

Total Protein = 5.9 gm.%

NO.	FILTRATE pH	APPROX. SERUM Mg MG.%	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	pK Ca PROT.
12A	7.32	2	8.2	5.8	71	1.73
12B	7.29	3	8.3	5.9	71	1.74
12C	7.31	4	8.0	5.8	73	1.70
12E	7.33	6	8.1	6.0	74	1.67
12F	7.29	7	7.9	5.9	75	1.65
12H	7.32	20	8.5	6.5	76	1.61
12G	7.30	28	8.5	6.7	79	1.56

In numbers 12A through 12F increasing amounts of a $MgCl_2$ solution containing 32.5 mg. per 100 cc. magnesium were added to 21 cc. aliquots of serum. In numbers 12H and 12G a 325 mg. per 100 cc. magnesium solution was used. Each sample was then diluted to 25 cc.

$MgCl_2$ to serum beyond any presumed physiological or clinical pathological range (Table XVIII).

An approximate serum magnesium range of 2 mg.% to 28 mg.% was ob-

tained. The total serum calcium concentration averaged 8.2 mg.% and the total protein concentration was approximately 5.9 gm.%. The percentage of ultrafilterable calcium increased only moderately with increasing serum magnesium concentration and the increase in percentage was insignificant within the physiological serum magnesium range.

This experiment was repeated and magnesium determinations were made to see whether this ion would behave similarly to calcium when present in excess. As may be seen in Table XIX there were fluctuations in percentages of both ultrafilterable calcium and magnesium with increasing magnesium concentration, but these fluctuations were similar in direction and degree to each other and are probably related to the unavoidable variation in pH. In the serum magnesium range of 2.2 to 26.4 mg.% and the pH range of 7.28 to 7.46, magnesium is found to be approximately 83% ultrafilterable. This is somewhat

TABLE XIX

Effect of Increased Serum Mg on Ultrafilterable Ca and P

Total Protein = 6.4 gm.%

NO.	FILTRATE pH	SERUM Mg MG.%	FILTRATE Mg MG.%	% FILT. Mg	SERUM Ca MG.%	FILTRATE Ca MG.%	% FILT. Ca	SERUM P MG.%	FILTRATE P MG.%	% FILT. P	1 GM. PROT. BINDS	
											mg. Ca	mg. Mg
18A	7.43	2.2	1.7	77	7.8	5.2	67	4.3	4.7	109	0.44	0.09
18E	7.28	9.4	8.0	85	7.8	6.1	78	4.3	4.6	107	0.30	0.26
18F	7.36	16.1	13.8	86	7.9	6.0	76	4.3	4.7	109	0.34	0.46
18D	7.46	26.4	21.7	82	7.8	5.4	69	4.3	4.3	100	0.41	0.88

To 14 cc. aliquots of a single pool of serum were added increasing amounts of a $MgCl_2$ solution containing 325 mg. magnesium per 100 cc. The samples were then ma .p to 15 cc. with distilled water.

higher than the 75% found by Watchorn and McCance (30) in human serum without controlled pH and also higher than the 73% found by Greene and Power (31) who used vividialysis in the dog. As in the experiment seen in Table XVIII, no significant effect of the increased serum magnesium is reflected in the percentage of ultrafilterable calcium. No evidence of an increased amount of non-filterable phosphorus is found until a serum magnesium of 16.1 mg.% is exceeded.

The amounts of calcium and magnesium combined with protein at each of these levels of serum magnesium were calculated by the method shown below Table IX. With constant calcium concentration and increasing magnesium concentration, the amount of magnesium "bound" with protein rises proportionately to the total serum concentration of magnesium. The amount of calcium "bound" to protein remains relatively constant. The minor variations seen are probably related to the pH at which the ultrafiltration was carried out. From this experiment it is concluded that the normal serum proteins have

the capacity to "bind" relatively large amounts of magnesium without seriously impairing their ability to "bind" calcium.

DISCUSSION

The studies here reported have to do with calcium, protein and phosphorus relationships in pooled *normal human serum, the protein of which is quantitatively and qualitatively normal and with normal concentration of the major electrolytes*. The criterion of the status of the calcium-phosphorus-protein relationships is based upon the ultrafiltration of calcium and phosphorus through a cellophane membrane under 200 mm. Hg pressure, at physiological pH and under anaerobic conditions. As with all such ultrafiltration techniques, while the experiment continues, the protein and calcium concentrations in the residue rise, whereas the concentration of water falls. However, the concentration of calcium in the small volume of ultrafiltrate obtainable at 16 hours is the same as that in the larger volume which is present at 24 or 48 hours; and this fact strengthens the belief that the calcium-protein relationship is unchanged during the ultrafiltration procedure. Furthermore, sodium, chloride, and potassium are found at concentrations reflecting the Donnan equilibrium effect on the two sides of the membrane. From these studies no conclusions can be drawn as to the "state" of the calcium which is filterable, i.e. whether it is all ionized or not.

Under the conditions of these experiments, the calcium of normal serum is found to be approximately 70% ultrafilterable—i.e., with total serum calcium 10 mg. per 100 cc., the calcium concentration of the ultrafiltrate is 7 mg. per 100 cc. Additions of sodium, potassium and magnesium, in quantities such that physiological ranges are greatly exceeded, do not affect the status of the calcium-protein-phosphate relationship as judged by the ultrafiltration. The magnesium of serum is found to be approximately 83% ultrafilterable. Since magnesium is a divalent cation as is calcium, it might be expected to bear the same relationship to protein as does calcium. The fact that magnesium in physiological pH range has been said to exist mainly as $Mg(OH)^{+}$ rather than Mg^{++} (32) may account for its different behavior from calcium.

When the calcium concentration of normal serum is increased by adding calcium salts in varying quantities, measurement of the calcium of ultrafiltrate and residue discloses that there is an increased amount of calcium proteinate as well as increase in the ultrafilterable calcium in degree such that a relatively constant proportion between the two is maintained. From these data a pK for calcium proteinate may be derived which is relatively constant for each pool of serum at a given pH. The pK values derived from our data are significantly lower than those derived by McLean and Hastings (20) who used the frog heart method, and also lower than those calculated by McLean and Hastings

(20) from the work of other investigators who used other ultrafiltration techniques in which the pH of the serum was not strictly controlled (33, 34), as well as the *in vivo* dialyzing system in which pH was controlled (31).

All but an insignificant fraction of inorganic phosphate in normal serum is ultrafilterable. When, however, calcium is raised to 20 mg.% or higher in normal serum, there is found a sharp fall in the phosphorus concentration of the ultrafiltrate and usually the concentration of calcium in the ultrafiltrate falls likewise. It seems possible that at these concentrations some sort of colloidal calcium-phosphorus "complex" is formed, which does not pass the ultrafilter, perhaps because "bound" to protein.

If the concentration of inorganic phosphorus is raised in slightly diluted normal serum, no evidence of increase in this hypothetical colloidal calcium-phosphorus complex formation is discernible even at levels of phosphorus as high as 30 mg.% (see Table XII). However, when calcium is maintained at normal concentration of 10 mg.%, serum phosphorus may be raised only to 17 mg.%, when a fall in the "% filterable calcium" suggests such a complex formation.

When one begins with a serum with either calcium or phosphorus concentration elevated above the norm, and then raises the concentration of the other, there is found, as might be expected, an increase in the percentage of non-filterable calcium and phosphorus at lower concentrations of both calcium and phosphorus than is the case if either constituent is within normal range.

To normal individuals calcium gluconate has been administered intravenously over periods of four hours so that serum calcium is raised to 14 or 15 mg.%. Serum taken from such persons and subjected to ultrafiltration has shown results identical with those obtained when calcium has been added to serum *in vitro* (35). Pathological sera, *with normal protein content*, but with elevated calcium or phosphorus (such sera being obtained from patients with hyperparathyroidism, sarcoidosis, poisoning with vitamin D, skeletal cancer or renal insufficiency), have also exhibited exactly the same behavior when subjected to ultrafiltration as have the normal sera to which calcium and phosphorus have been added *in vitro* (35).

Any clinical implications drawn from these experiments must be approached warily. As previously mentioned, the experimental ultrafiltration pressure is far higher than that to which any capillary is subjected; and the protein on the serum side of the membrane is constantly increasing to a degree not met in the physiological situation. In the animal organism, portions of the extracellular fluid are constantly being equilibrated with calcium which is resident on the vast surfaces of the skeletal lime salts (32), which must serve as a buffering mechanism to take up or provide calcium and maintain serum calcium as "one of nature's physiological constants" (1).

Despite these wide divergences between the *in vitro* and *in vivo* conditions,

it does seem apparent that there is no chemical situation inherent within the serum itself such that when calcium concentration is raised, inorganic phosphorus must fall, or vice versa. If serum phosphorus rises as the result of renal insufficiency, calcium may fall (actually it is rarely found much lowered), but the fall is a result of a physiological mechanism and not "supersaturation of the serum with calcium and phosphorus," as is so often implied in the clinical literature.

One cannot help but wonder what happens *in vivo* when serum concentrations of calcium and phosphorus are reached which *in vitro* would result in reduced ultrafiltration of calcium and phosphorus. So-called parathyroid "intoxication" in animals and man results when serum calcium reaches 20 mg.% or higher (36, 37, 38). There is usually rapid rise in serum phosphorus, renal shutdown, circulatory collapse and death. *In vitro*, when serum phosphorus is normal, a calcium concentration of 20 mg.% or higher results in sharp reduction in ultrafilterability of calcium and phosphorus.

SUMMARY

Pooled normal human serum has been subjected to ultrafiltration anaerobically and with controlled pH at 200 mm. Hg pressure. Failure to saturate the serum with 5% CO_2 in oxygen results in sharp variations in the ultrafilterable calcium. Freezing and later thawing does not alter results, provided pH is adjusted. Additions of potassium, sodium and magnesium to serum so as to result in concentrations of these elements far above physiological ranges do not affect the ultrafilterability of calcium. Sodium, potassium and chloride ions were found to be wholly ultrafilterable. A small and inconstant fraction of the inorganic phosphate was not ultrafilterable. Serum magnesium was found to be approximately 83% ultrafilterable.

With protein and phosphorus in the normal ranges, calcium added to normal serum results in an increase in calcium proteinate as well as ultrafilterable calcium. From these data a pK may be derived for calcium proteinate, yielding evidence that the calcium-protein relationship is governed by the law of mass action. At calcium concentrations of 20 mg.% or greater, the ultrafiltration of inorganic phosphorus, which up to this point has been nearly completely ultrafilterable, falls; and it is postulated that a calcium-phosphorus "complex" has been formed which is "attached" to protein.

Elevation of inorganic phosphorus of the serum results in decreased filterability of calcium and phosphorus at lower concentrations of calcium.

Certain clinical inferences are cautiously drawn.

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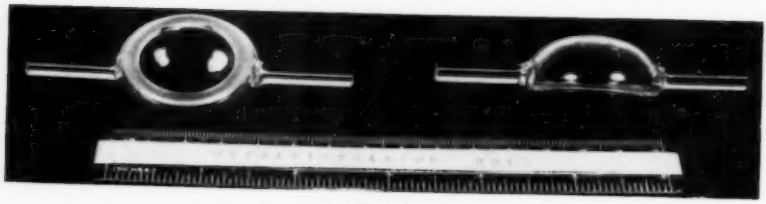

FIG. 1

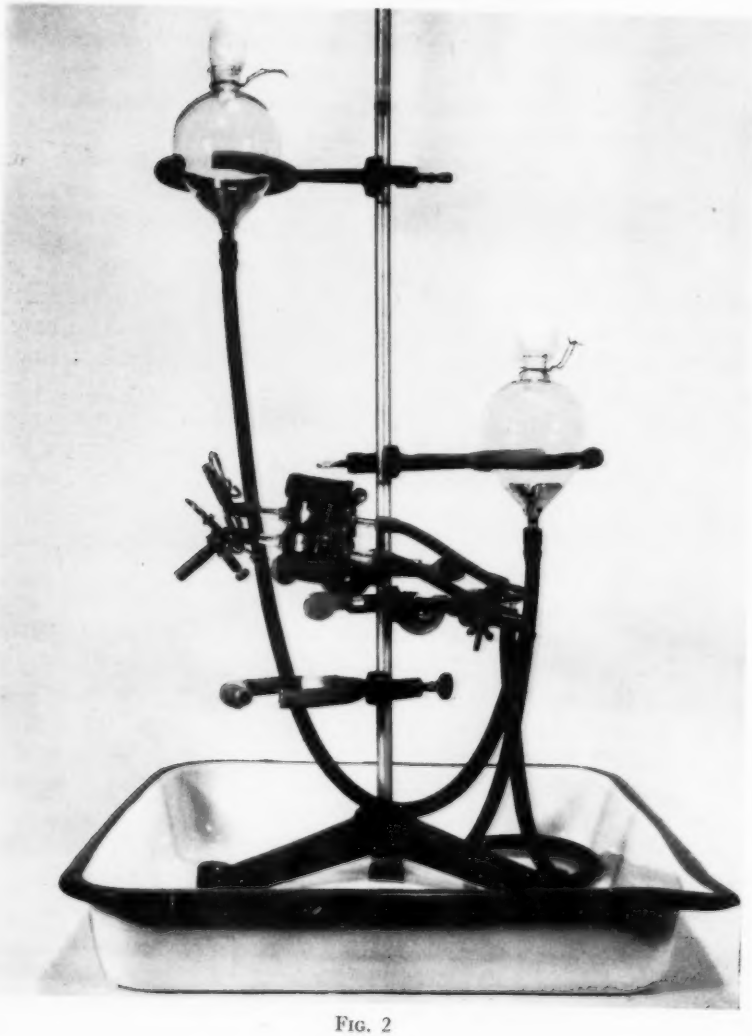

FIG. 2

APPENDIX

Description of method (12)

The apparatus for obtaining serum ultrafiltrates consists of two matching pyrex chambers*, each with two capillary sidearms (Fig. 1). DuPont cellophane #300-PT constitutes the ultrafiltration membrane and is prepared by soaking in distilled water for from one to 72 hours. When the apparatus is to be assembled, the excess moisture is removed from a single thickness of cellophane by pressing it between filter papers. The cellophane is then placed smoothly over the open face of one of the chambers and the two chambers are clamped tightly together with a rubber-jawed clamp. To load the apparatus the chambers are placed in the "vertical" position. To the upper sidearm of each is attached a short rubber tube and to the lower sidearm of each is attached a rubber tube 40 cm. in length connected to a leveling bulb containing mercury. Mercury is allowed to completely fill the chambers as well as the short rubber tubes and a glass capillary introductory tube which is temporarily attached to the left hand tube. The right hand tube is then clamped off. The introductory tube is placed beneath the surface of the serum to avoid any introduction of air and the left hand leveling bulb is lowered sufficiently to create negative pressure in the left hand chamber. When sufficient serum (usually 8 to 10 cc.) has entered the chamber, the left hand tube is clamped off. The chambers are tilted to the left about 135° and the left hand rubber tube is squeezed to replace the serum in this sidearm by mercury, thus producing a mercury seal. The chambers are now placed at a 15° angle from the horizontal. The pressure is adjusted to a suitable gradient by raising the left hand leveling bulb to the desired height above the right. Ultrafiltration now proceeds (Fig. 2). At the appropriate time both ultrafiltrate and residue may be removed by bringing both leveling bulbs to approximately 12 cm. above the chambers, placing the chambers again in the "vertical" position and unclamping the short rubber tubes to which are connected two graduated 10 cc. pipettes. The ultrafiltrate and residue are collected and measured in the pipettes and pH determinations are immediately done.

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THE INFLUENCE OF CORTISONE ON THE EVOLUTION OF ACUTE INFECTION AND THE DEVELOPMENT OF IMMUNITY

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Numerous investigators have drawn attention to the role of the adrenal hormones in the pathogenesis of infection. Harvey Cushing, in his discussion of pituitary basophilism, a condition associated with an excessive production of cortical hormone (1), emphasized the hypersusceptibility of patients with this malady to infection. In patients with Addison's disease and in adrenalectomized animals, increased susceptibility to stress conditions, including infection, has been observed (2). During the progress of the present study, many reports have appeared concerning the influence of cortisone on the course of clinical and experimental infections. Finland and co-workers (3) have shown that ACTH therapy in pneumococcal pneumonia may result in a dramatic clinical improvement despite the persistence of a considerable bacteremia. A marked enhancement of blood and tissue infection by streptococci (4, 5) and pneumococci (6) has been observed in rabbits following treatment with cortisone; and in experimental tuberculous infections in rats (7), guinea pigs (8), and mice (9) cortisone has intensified the infection. Shwartzman (10) has reported a marked acceleration of poliomyelitis in mice and an extraordinary increase of susceptibility to this infection in hamsters by cortisone.

In previous observations on the influence of cortisone on the Arthus reaction (11), a greater incidence of death from diarrhea of presumably an infectious nature was noted in the treated animals, and it was suggested that cortisone might have increased the susceptibility of the laboratory animals to intercurrent infection. Following this observation, the present studies were undertaken to determine the effect of cortisone on the pathogenesis of acute infection and the development of immunity.

METHODS AND MATERIALS

Cortisone, obtained from Merck and Company as a saline suspension of cortisone acetate, was administered to rabbits in a single daily intramuscular injection.

White male rabbits (New Zealand strain), weighing approximately 2 kg. were employed in studies on cutaneous and blood infection, while larger rabbits weighing 3 to 4 kg. were used in experiments on testicular infection. In each case, an 18 hour culture of pneumococcus type II, grown on Walker's modified broth medium with 0.1 per cent glucose and 1 per cent

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normal rabbit serum was used as the test organism. The culture was obtained from the heart's blood of a mouse infected 6 hours previously. The pneumococcus was passed daily through mice in order to maintain its virulence. The culture was counted in a bacterial chamber and this count was verified by the pour-plate technique. One thousand organisms contained in 0.2 ml. of broth were injected into the skin for the production of cutaneous infection. For the establishment of a primary blood infection, rabbits were inoculated into the marginal ear vein with 1 billion organisms (approximately 2 ml. of the original culture). At varying intervals after infection, 0.5 ml. of blood was drained from the opposite ear and expelled into either nutrient broth or agar. The number of organisms in the blood sample was estimated by serial dilution of the blood in blood-agar and plating. Plates were incubated at 37° C for 24 to 48 hours and then counted. Although this organism (grown under the conditions herein described) was highly virulent for mice, over 70 per cent of rabbits survived following an intravenous injection of approximately 1 billion organisms.

For the production of testicular infections in rabbits, 1,000 pneumococci type II from an 18 hour culture were injected into each testis. After establishment of the infection, the testes were removed singly so that specimens were obtained from each rabbit after two different time intervals. Each testis was immediately placed in a Waring blendor with 50 ml. of broth and homogenized and within 2 minutes pour plates were prepared to obtain the bacterial count of the suspension.

In order to compare the effects of cortisone on an infection produced by a more virulent organism, a pneumococcus type I organism was also employed for intracutaneous infection. This organism was grown for 6 hours on beef heart infusion broth with 0.2 per cent glucose and 2 per cent defibrinated rabbit blood. Two other organisms were also employed to determine the effect of cortisone treatment on the rate of clearance of organisms from the blood stream; a strain of rough pneumococcus (DR1) grown on modified Walker's medium with added glucose and rabbit serum, and a strain of hemolytic *Staphylococcus aureus* grown on beef broth with glucose and rabbit serum.

In a study of immune response in rabbits treated with cortisone and inoculated intravenously with pneumococci, the otherwise lethal infection was interrupted by the use of procaine penicillin in oil.

A pooled typhoid vaccine containing 1 billion organisms per ml. was used to determine the effect of cortisone on the pyrogenicity and toxicity of heat-killed typhoid bacilli.

I. THE ACTION OF CORTISONE IN PNEUMOCOCCAL INFECTIONS

A. The effect of cortisone on cutaneous and testicular pneumococcal infections

Five rabbits injected intramuscularly with cortisone at a dosage of 15 mg. per kg. for 1 day prior to infection and at 10 mg. per kg. per day thereafter. These animals, as well as 5 controls, were infected intracutaneously with approximately 1,000 pneumococcus type II organisms. The onset and size of the skin reaction, the degree of bacteremia, and the subsequent febrile response were observed and are recorded as in Tables I, II, and III and presented graphically in figure 1. The size of the skin response is given as the product of its width, length, and height measured in centimeters.

In the control animals, pink edematous lesions were present at the site of inoculation as early as 12 hours after infection. For the next 24 hours the erythema and edema increased and in 3 of 5 animals, the lesions became

TABLE I

Effect of Cortisone on Skin Lesions produced by Local Pneumococcus Type II Infection

RABBIT NUMBER	TREATMENT	MAGNITUDE* OF SKIN LESION PRODUCED BY INOCULATION OF 1,000 Pn II				
		12 hrs.	24 hrs.	36 hrs.	48 hrs.	72 hrs.
1	Cortisone, 15 mg./kg, 1 day prior	0 pp†	3.0 p	6.6 p	10 p	3.6 pp
2	to infection; 10 mg./kg, per day	0 pp	0 pp	0.1 pp	0.5 p	1.4 p
3	thereafter	0 pp	5.4 p	4.3 p	5.4 r	
4		0 pp	0.1 p	4.5 p	3.0 p	4.5 p
5		0 pp	0 pp	0 pp	0 pp	0.2 p
Median		0	0.1	4.3	3.0	3.6
6	Control	13 p	9.0 h	22 r	27 r	2.1 r
7		4.2 p	3.4 h	8.0 r	16 r	3.0 h
8		0.8 pp	2.7 p	5.9 r	5.8 r	6.2 r
9		0.8 pp	1.8 h	3.5 r	3.4 r	3.8 r
10		0.1 pp	1.4 p	2.2 r	3.2 r	3.2 r
Median		0.8	2.7	5.9	5.8	3.8

* Product of length, width and height measured in centimeters. pp—pale pink, p—pink, r—red, h—hemorrhagic.

† Flat erythema.

TABLE II

Effect of Cortisone on Bacteremia Following Cutaneous Infection with Pneumococcus Type II

RABBIT NO.	TREATMENT	BACTERIAL COUNT, COLONIES/ML. BLOOD AT							
		12 hrs.	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.	144 hrs.	192 hrs.
1	Cortisone 15 mg./	12	48	28	>1,000	>1,000	>1,000	Dead	
2	kg, 1 day prior	0	0	5	116	626	>1,000	Dead	
3	to infection; 10	20	8	72	>1,000	>1,000	Dead		
4	mg./kg, per	20	156	60	23	356	>1,000	16	>1,000(S)*
5	day thereafter	56	200	80	28	>1,000	Dead		
Median		20	48	60	116	>1,000	>1,000		
6	Control	40	220	4	>1,000	>1,000	262	0	0(S)
7		8	16	24	10	2	18	4	0(S)
8		16	60	20	0	2	58	0	2(S)
9		24	72	188	6	12	8	0	16(S)
10		168	80	28	0	10	50	2	2(S)
Median		24	72	24	6	10	50	0	2

* Cortisone treatment discontinued after 96 hours.

(S) Survived.

hemorrhagic. In contrast, at 12 hours the cortisone-treated animals showed only a flat, pale pink reaction at the site of infection and only after 24 to 36

hours did the lesions of the treated animals become as prominent as the controls.

A moderate bacteremia appeared in both groups of animals beginning at 12 hours. Small numbers of organisms persisted in the blood of the control animals until the sixth day, at which time 3 of 5 animals gave negative blood cultures. All 5 of the controls survived. However, in the animals treated with cortisone, there was a progressive bacteremia and by the sixth day of infection 4 of 5 animals had succumbed. The sole survivor had a severe septicemia at the time treatment with cortisone was discontinued.

TABLE III

Effect of Cortisone on Febrile Response Following Intracutaneous Inoculation of Pneumococcus Type II

RABBIT NO.	TREATMENT	RECTAL TEMPERATURE DEGREES CENTIGRADE							
		0	12 hrs.	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.	144 hrs.
1	Cortisone 15 mg./kg, 1 day prior to infection; 10 mg./kg, per day thereafter	39.2	39.7	39.5	40.2	40.2	41.5	41.5	Dead
2		39.1	39.2	39.5	39.4	39.4	39.4	40.4	Dead
3		39.2	39.6	39.6	40.2	40.8	40.9	Dead	
4		39.6	39.7	39.4	39.8	39.7	40.0	40.3	40.0
5		39.2	39.7	39.4	39.6	39.5	41.0	Dead	
Average		39.3	39.6	39.5	39.8	39.9	40.6	40.7	
6	Control	39.1	39.7	40.5	41.2	40.3	41.0	41.1	40.2
7		39 1	39.8	40.1	39.8	39.5	40.1	40.5	40.1
8		39.5	39.9	39.9	39.6	39.7	39.5	39.5	39.4
9		39.4	39.8	40.1	39.9	40.0	39.7	39.6	39.4
10		39.5	39.8	39.7	39.8	39.7	39.4	39.5	39.6
Average		39.3	39.8	40.1	40.1	39.8	39.9	40.0	39.7

As shown in Table III, the initial febrile response of the treated group of animals was suppressed. However, with increasing septicemia, the febrile response of the treated animals became progressively greater and eventually surpassed that of the controls.

To obtain further information on the effect of cortisone on local inflammation, a series of 12 similarly treated and infected rabbits and 12 infected controls were killed 24 and 48 hours after infection for histopathological study. Striking differences in the cellular inflammatory responses were observed between these two groups of animals. In the skin of the control animals, there were numerous neutrophilic leukocytes, necrosis and thrombosis of small blood vessels, hemorrhage and edema (fig. 1a). In the treated animals, the leukocytic response was much less prominent and the blood vessels were entirely normal (fig. 2a). In the control group, bacteria were rarely observed; whereas, in the

cortisone-treated animals, numerous bacteria were present (fig. 3a). It has been shown that cortisone produces a lymphocytopenia and an increase in the absolute number of circulating polymorphonuclear leukocytes (12). The decreased number of leukocytes at the inflammatory site was therefore due to an interference with the migration rather than a paucity of the circulating

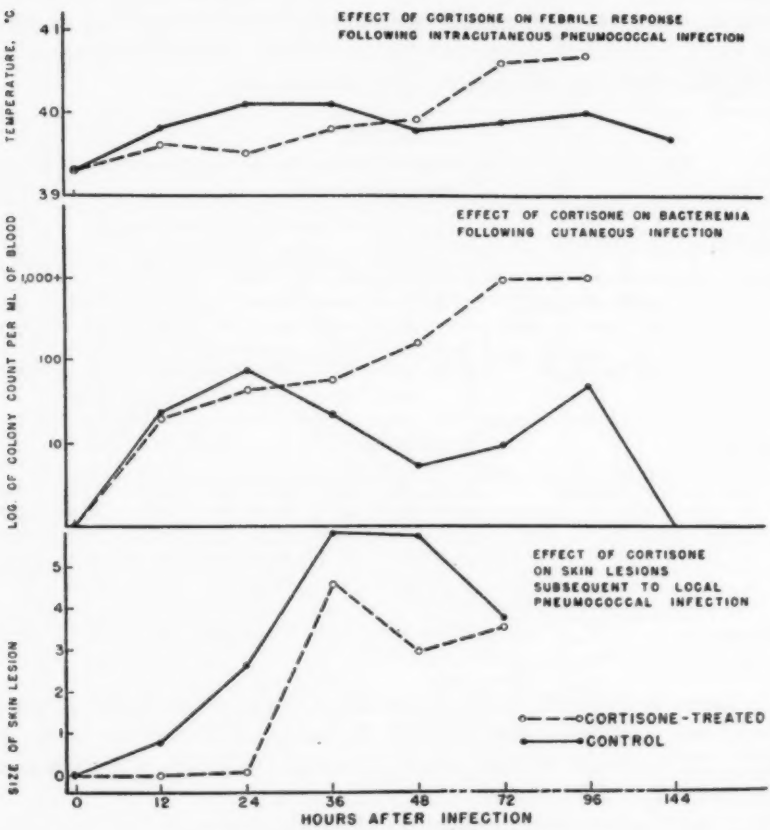

FIG. 1. After data of Tables I, II and III

cells. Since phagocytosis of bacteria was observed in the treated animals, no evidence was obtained that cortisone had destroyed the phagocytic capacity of the individual leukocytes.

The preceding histological observations suggested that cortisone favored the local growth of bacteria in tissue. To study this phenomenon quantitatively, rabbits, weighing 3 to 4 kg. were injected intratesticularly with approximately

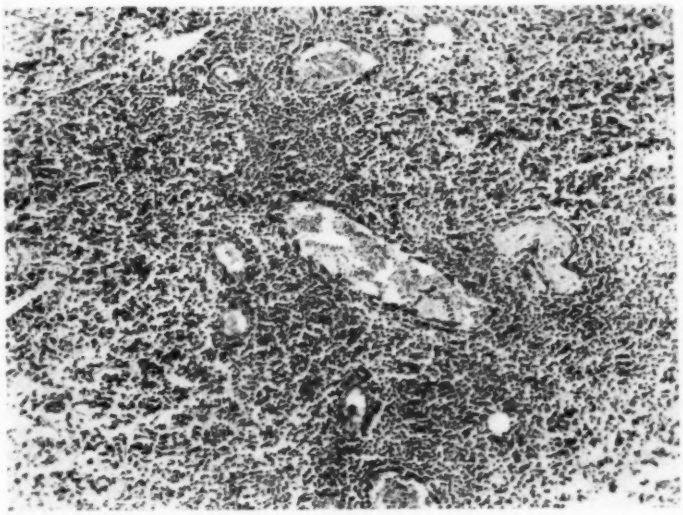

FIG. 1a. Numerous polymorphonuclear leukocytes in skin of a control rabbit. No bacteria are present. (100×)

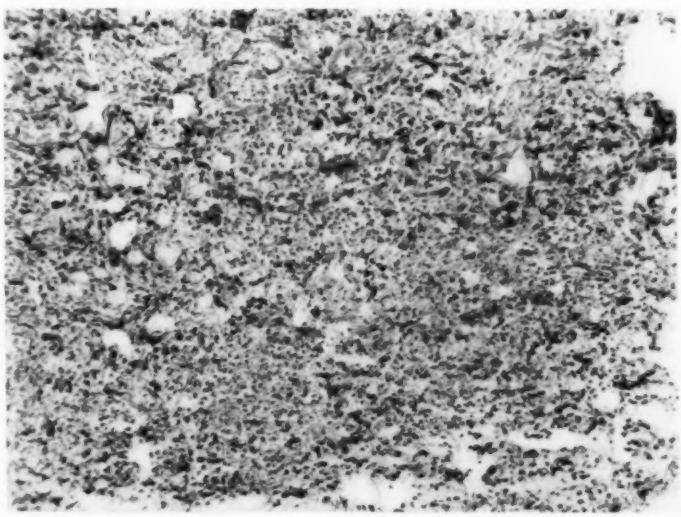

FIG. 2a. Lesion of a cortisone-treated rabbit showing scanty cellular exudate and fragmentation of collagen of derma. (100×)

1,000 pneumococcus type II organisms. Half of the animals were treated for 4 days prior to infection with successive daily doses of 50, 40, 20, and 20 mg. of

cortisone and then received 20 mg. of cortisone per day. The infected testes were removed after varying intervals and the number of organisms determined as previously described. At the same time, a blood culture was made by the pour-plate technique.

Although there was considerable variation in the response of individual rabbits to the infection (Table IV, fig. 2), in general the number of organisms at the site of infection in the controls increased a thousand fold within the first 24 hours. Thereafter, there was a decrease so that after 72 hours most of the testes were free of bacteria. The moderate bacteremia which developed took a course parallel to the tissue infection, and the blood of most of the

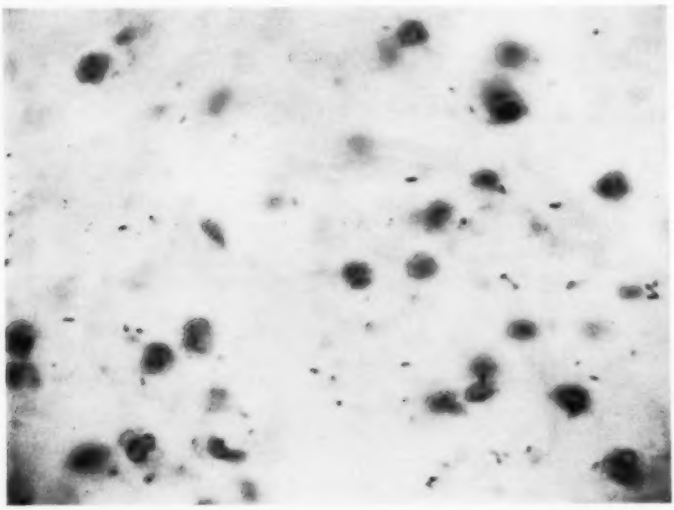

FIG. 3a. Numerous bacteria in skin of cortisone-treated rabbit. Gram-Weigert stain of lesion in Fig. 2a. (1400×)

The photomicrographs were made by Mr. K. Cramer Lewis, Department of Illustration, Washington University School of Medicine. Comparable sections were obtained from animals inoculated intradermally 24 hrs. previously with approximately 1,000 pneumococci Type II.

animals was negative by the third day. The cortisone-treated animals reacted quite differently. Ten hours after infection the number of organisms per testis was 30-fold greater than in the controls. The number of organisms and the bacteremia continued to increase, so that by 48 hours there was massive infection of the testis and blood. Despite the height of the bacteremia, a comparison of the blood and tissue counts indicated that in many of the animals there was a greater growth of organisms in the testis than could be accounted for by

TABLE IV

The Effect of Cortisone on Intratesticular Pneumococcal Infection in Rabbits

TIME IN HOURS	CORTISONE		CONTROL	
	No. of Organisms in testis	No. of Organisms per ml. blood	No. of Organisms in testis	No. of Organisms per ml. blood
0			2,100	
			2,000	
			700	
			600	
			2,500	
			2,200	
			1,500	
			1,400	
Median			1,700	
5	8,300		8,300	
	7,600		2,300	
	11,000		5,300	
	17,000			
	21,000			
Median	11,000		5,300	
10	350,000	178	21,000	8
	12,000		12,000	
	950,000	4	15,000	4
	9,900		36,000	
	380,000	2	210,000	
	330,000	20	31,000	0
	770,000	0	140,000	56
	2,200,000	78	32,000	0
			320,000	84
			52,000	2
			600	0
Median	365,000	12	32,000	3
24	22,000,000	13,000	15,000	216
	35,000,000		2,300,000	
	180,000	420,000	460,000	50
	900,000		8,600,000	2,700
	150,000	2	36,000,000	2
	39,000	136	1,100	2
	2,600,000			
	410,000	0		
	37,000,000	9,500		
	2,300,000	40		
	1,800,000	37,000		
Median	1,800,000	4,800	1,400,000	50

TABLE IV—*Continued*

TIME IN HOURS	CORTISONE		CONTROL	
	No. of Organisms in testis	No. of Organisms per ml. blood	No. of Organisms in testis	No. of Organisms per ml. blood
48	13,000,000	9,600,000	110,000	8
	3,400,000		13,000	
	320,000	2,900	6,000	720
	58,000,000	48,000,000	24,000	
	8,100,000	15,000,000	34,000	44
	2,800,000,000	260,000	300	0
	920,000,000	100,000,000	1,500,000	15,000
			0	0
Median	13,000,000	12,300,000	13,000	8
72	Dead		0	0
	Dead		0	0
	Dead		0	0
	33,000,000	440,000	0	0
	230,000,000	3,300,000	190,000,000	1,400
Median			0	0

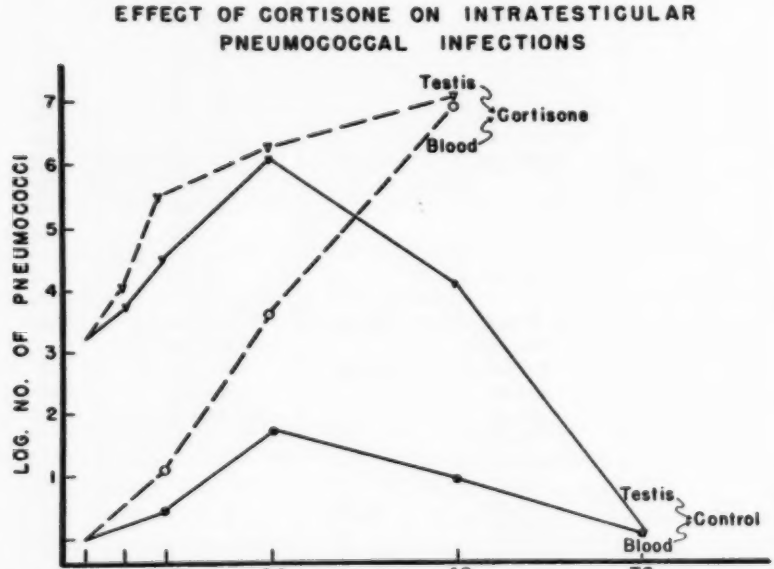

FIG. 2. After data of Table IV

the numbers in the blood stream. At 72 hours after infection, 3 of 5 animals had succumbed.

A primary result of inflammation is the limitation of the spread of infection and dilution of noxious agents. A temporary suppression of inflammation by cortisone might therefore lead to a more extensive tissue damage by the invading organism and result in a subsequent reaction greater than is ordinarily

TABLE V

Effect of Cortisone on Intracutaneous Pneumococcal Type I Infections in Rabbits

RABBIT NO.	TREATMENT	SIZE OF LESION		COLONY COUNT/ML. BLOOD		TIME OF DEATH HOURS
		12 hrs.	24 hrs.	12 hrs.	24 hrs.	
1	Cortisone, 30, 20, 10 mg. prior to infection and 10 mg. per day thereafter		3.4 x 9.5 x 0.25 pp.		1.1×10^5	48
2			6.0 x 10 x 0.35 h		1.4×10^6	48
3		4.1 x 5.5 x 0.1 pp.	7.8 x 13 x 0.25 h, with extensive abdominal edema	1.9×10^3	2.7×10^5	48
4		3.0 x 3.4 x 0.05 pp.	6.0 x 15 x 0.4 h, with extensive abdominal edema	1.1×10^4	5.4×10^6	48
5		3.7 x 5.6 x 0.15 pp.	9.0 x 15 x 0.25 h, with extensive abdominal edema	50	4.4×10^4	48
6	Control		4.6 x 12 x .25 p.		1.7×10^4	48
7			8.5 x 10 x 0.4 p.		1.3×10^4	94
8		2.0 x 3.9 x 0.15 p.	2.5 x 6.0 x 0.2 h.	18	0	Lived
9		3.5 x 6.5 x 0.2 p.	5.0 x 13 x 0.3 h.	30	48	Lived
10		3.4 x 5.2 x 0.15 p.	4.2 x 10 x 0.2 p.	2.6×10^3	44	Lived

B. The effect of cortisone on pneumococcal bacteremia

observed without treatment. Such was the case in intracutaneous infections produced by a virulent type I pneumococcus as indicated in Table V. Here, the prominent feature of the treated animals was the presence of extensive edema that migrated downward into the abdominal wall.

The marked septicemia which followed infection in the cortisone-treated animals warranted further study. A group of 16 rabbits were injected intravenously with 1 billion pneumococcus type II organisms and the subsequent blood stream infection followed. Eight of these rabbits received successive doses of 20, 10 and 10 mg. of cortisone at 72, 48, and 24 hours prior to infection and 10 mg. per day thereafter. The results are recorded in Table VI and are shown graphically in figures 3 and 4. In the control animals, the organisms

TABLE VI

Removal of Pneumococci from the Blood of Normal and Cortisone-Treated Rabbits[1]

RABBIT NO.	TREATMENT	NUMBER OF ORGANISMS (IN THOUSANDS) PER ML. OF BLOOD										
		0	1 hr.	2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.
1	Cortisone, 20	2,600	900	1,000	2,100	100	160	3,300	3,300	9,700	Dead	
2	mg., 10 mg.	7,600	470	1,300	1,100	180	41	280		300	Dead[2]	
3	and 10 mg.	2,300	42	94	160	630	76	4.0	1.3	10	7.3	Dead
4	at 72, 48 and	4,700	1,100	2,300	14,000	2,200	16	3,300	8,400	28,000	Dead	
5	24 hrs. prior	4,000	1,600	5,200	9,200	6,800	160	23,000	21,000	12,000	Dead	
6	to infection;	2,300	2,100	2,400		30	17	110,000	930,000	Dead		
7	and 10 mg.	3,900	760	1,400	4,600	2,500	47	1,900	4,300	12,000	Dead	
8	per day thereafter	2,600	760	400	180		9.7	37	410	580		Dead
Median		3,200	830	1,400	2,100	630	44	2,600	4,300	9,700		
9		2,900	100	140	110	0.54	0.78	1.8	1.1	0.42	0.77	.005
10		1,900	180	290	6.0	0.090	2.0	14		0.15	0.16	0
11		3,600	720	360	35	37	160	4.7	0.60	0.52	0.13	0
12	Control	3,300	6.0	1.9	0.36	1.3	8.8	43	7.0	3.5	0.59	0.15
13		5,600	1,300	750	8.2	1.3	3.5	1.6	5.5	1.0	0.14	.004
14		2,400	1,300	500	2.9	0.29	7.0	17	4.2	2.6	0.024	0
15		4,000	720	360	22	1.6	8.0	1.8	.014	.076	0.024	.010
16		4,800	2,200	7,200	8,200	52	41	40	1,100	22,000	Dead	
Median		3,500	720	360	15	1.3	7.5	9.4	4.2	0.76	0.13	.004

[1] Rabbits injected intravenously with 1 billion Type 2 pneumococci.

[2] Death due to accidental trauma.

were removed very rapidly from the blood, so that by the sixth hour the number had been reduced a thousand fold. After a slight increase in the number of circulating organisms between the 6th to 24th hours, there was a constant

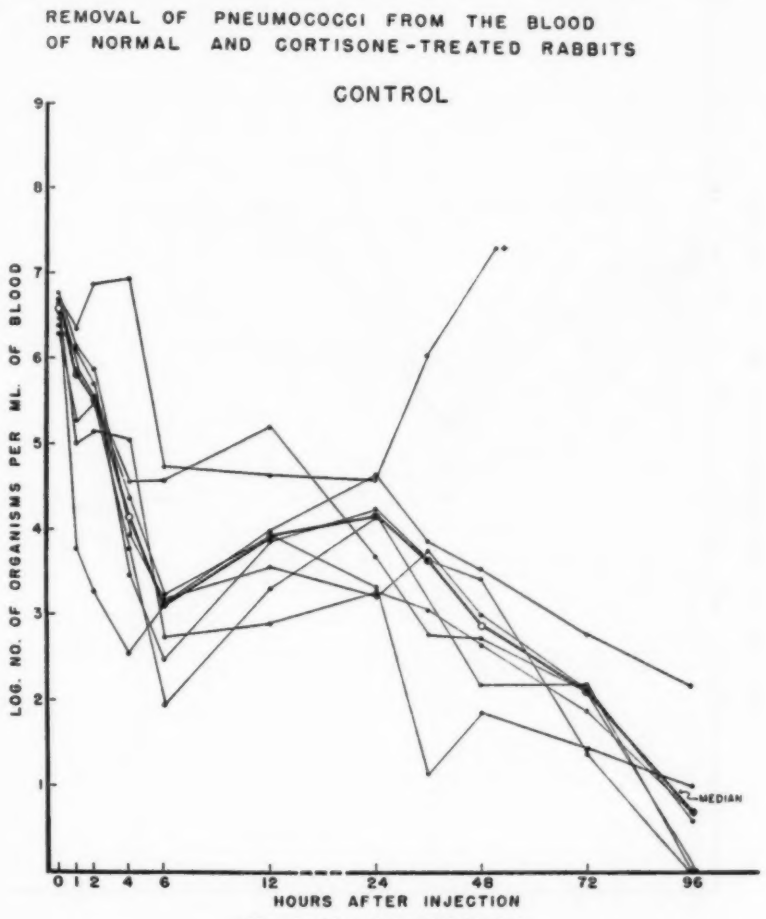

FIG. 3. After data of Table VI

and gradual clearing of the blood and by the fourth day, 3 of the 8 animals gave negative cultures. In one of the controls (# 16), which subsequently succumbed, the course of bacteremia was quite different and resembled that observed in the cortisone-treated group.

In the cortisone-treated animals, the initial fall in the number of organisms during the first 2 hours paralleled that observed in the controls. Thereafter

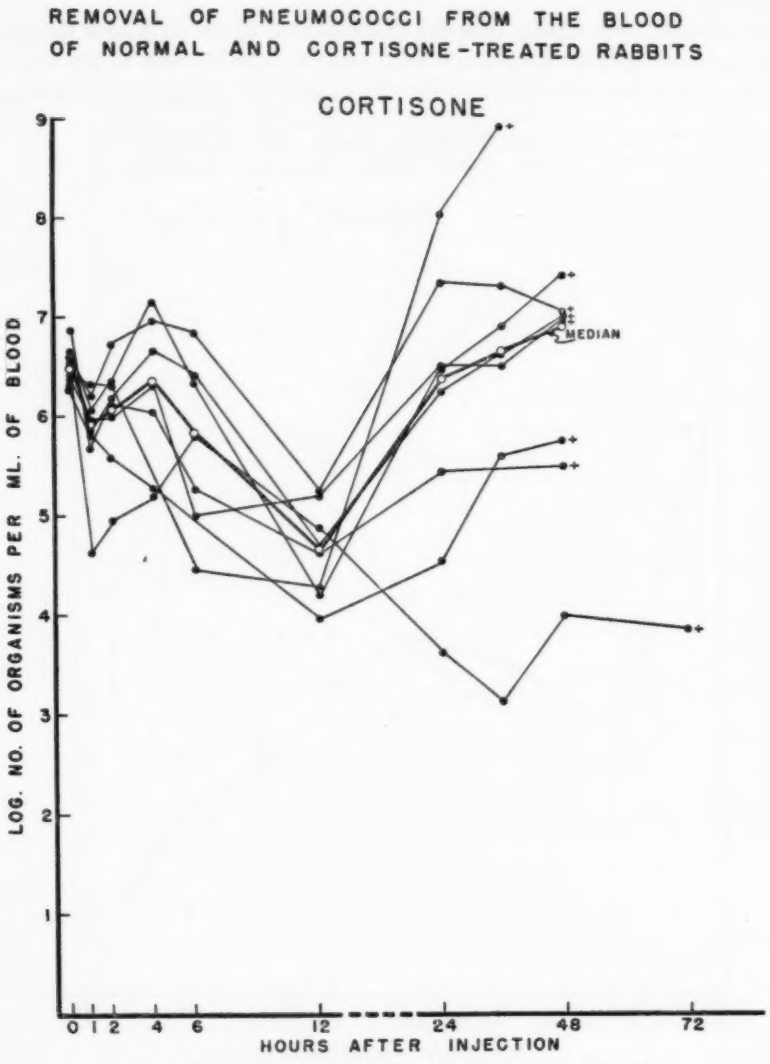
REMOVAL OF PNEUMOCOCCI FROM THE BLOOD
OF NORMAL AND CORTISONE-TREATED RABBITS
CORTISONE
MEDIAN
LOG. NO. OF ORGANISMS PER ML. OF BLOOD
HOURS AFTER INJECTION
0 1 2 4 6 12 24 48 72
1 2 3 4 5 6 7 8 9

FIG. 4. After data of Table VI. + denotes death of animal

the rate of clearance was quite different, and during the next 3 hours there was actually an increase in the bacterial count. However, this transitory increase was then followed by further removal of bacteria, so that by the twelfth hour the number of organisms in the blood of the treated group was only slightly more than that seen in the controls. After the twelfth hour, there was

TABLE VII

Effect of Cortisone on the Rate of Disappearance of Pneumococci from the Blood following Intravenous Infection[1]

RABBIT NO.	TREATMENT	NUMBER OF ORGANISMS (IN THOUSANDS) PER ML. OF BLOOD								
		0	1 hr.	2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.
1	Cortisone, 30 mg., 20 mg. and 10 mg. at 72, 48 and 24 hours prior to infection, and 10 mg. per day thereafter	11,000	4,100	4,000	1,400	110	120	7,200	Dead	
2		21,000	3,800	4,700	15,000	9,900	11,000	Dead		
3		9,500	3,600	3,600	30,000	300,000	1,000,000	Dead		
4		9,300	7,400	13,000	67,000	210,000	Dead			
5		9,500	2,500	4,200	14,000	12,000	1,800	180,000	Dead	
6		8,700	6,000	8,200	43,000	670,000	11,000	Dead		
Median		9,500	3,800	4,200	15,000	12,000	11,000			
7	Control	13,000	6,300	4,800	3,000	79	120	Dead		
8		11,000	3,200	2,900	2,200	320	79	Dead		
9		8,600	1,400	130	13	2.4	5.8	1.4	2.3	.24
10		1,400	2,500	2,800	360	6.0	23	77	12	.88
11		9,400	220	19	2.0	.36	3.0	.56	.32	0
12		13,000	1,400	1,400	59	1.4	7.0	3.7	1.3	.04
13		7,500	1,800	680	73	2.2	9.1	29	16	.12
Median		9,400	1,800	1,400	73	2.4	9.1	3.7	2.3	.12

[1] Rabbits injected intravenously with 1 billion Type 2 pneumococci.

an increasing septicemia and all 8 treated animals were dead by the fourth day after infection.

Essentially similar results were obtained in a second experiment, recorded in Table VII and presented in figure 5. Here the amount of cortisone administered to the animals was slightly greater than that employed in the previous experiment. The differences between the cortisone-treated animals and con-

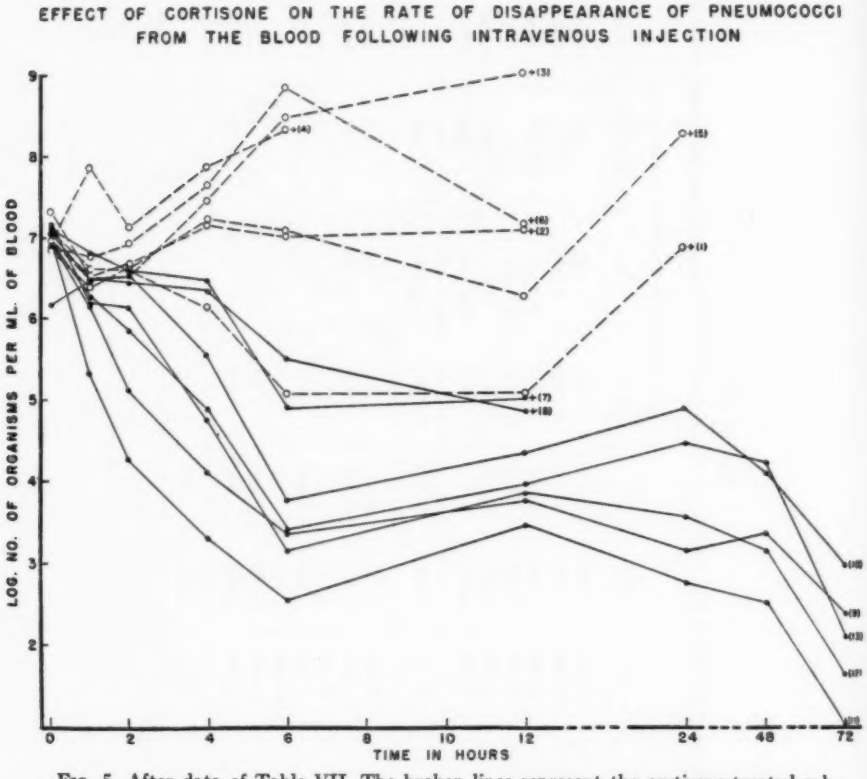

FIG. 5. After data of Table VII. The broken lines represent the cortisone-treated rabbits.

trols were even more striking. After an initial fall in the bacteremia in the first hour, there was in most of the treated animals an increase in the number of organisms for the next 5 hours. Thereafter the bacteremia was maintained at a high level with minor fluctuations until the animals succumbed to infection by the 24th hour. Two of the 7 control rabbits died of infection, and again these 2 showed the slowest initial clearance of organisms from the blood.

The course of bacteremia resulting from the encapsulated pneumococcus

type II is determined by at least two factors: the rate of growth of organisms in the blood, tissue fluids, and lymph, and their rate of removal and destruction by the phagocytic cells. In order to investigate the effect of cortisone on speed of removal of organisms from the blood in the absence of appreciable growth of the organisms, the blood clearance of an avirulent (rough) pneumococcus was measured. As is well known, avirulent cultures under ordinary circumstances are removed rapidly and show no evidence of proliferation in the blood stream. A group of 11 rabbits was injected intravenously with 1 billion organisms from an 18 hour culture of avirulent (DR1 strain) pneumococci. Six animals were treated with successive doses of 30, 20, 10, 10, and 10 mg. of

TABLE VIII

Effect of Cortisone on the Rate of Clearance of Unencapsulated Pneumococci (DR1 strain)[1]

RABBIT NO.	TREATMENT	COLONY COUNT/ML. OF BLOOD								
		1 min.	10 min.	20 min.	30 min.	1 hr.	4 hrs.	24 hrs.	48 hrs.	72 hrs.
1	Cortisone, 30, 20, 10, 10 and 10 mg., 5 days prior to infection; and 10 mg. per day thereafter	2.0×10^6	1.9×10^4	3200	748	36	12			
2		4.1×10^5	7.5×10^3	558	324	310	6	164	140	4
3		8.7×10^5	876	570	226	4	6	138	390	2
4		1.6×10^7	1.8×10^5	1900	1200	120	16	156	172	6
5		1.3×10^5	1.2×10^3	88	38	12	8	28	0	0
6		1.4×10^6	4.1×10^3	650	350	28	6	1000	1900	0
Median		1.4×10^6	7.5×10^3	650	350	36	8	156	172	2
7	Control	2.0×10^7	1.4×10^4	2200	732	44	80			
8		6.2×10^6	1.2×10^4	1500	780	236	6			
9		3.3×10^6	3.4×10^3	738	76	32	88	0	0	0
10		9.1×10^5	2.3×10^3	558	384	258	12	1400	680	0
11		9.0×10^5	516	116	48	12	22	32	10	116
Median		6.2×10^6	3.4×10^3	738	384	44	22	32	10	0

[1] Rabbits injected with 1 billion unencapsulated pneumococci.

cortisone for 5 days prior to infection and with 10 mg. of cortisone per day thereafter. The results are shown in Table VIII and figure 6. Unlike the slow removal following an injection of encapsulated bacteria, there was a rapid reduction of organisms from an estimated 10 million per ml. of blood to less than 100 in 1 hour. Cortisone had no effect on the rate of disappearance of these organisms.

Following passive immunization of rabbits with anti-pneumococcal rabbit serum, the rate of clearance of encapsulated pneumococci was similar to the clearance of unencapsulated pneumococci in normal animals. Previous treatment with large doses of cortisone had no effect on the clearance in these immunized animals. The results of this experiment are shown in Table IX.

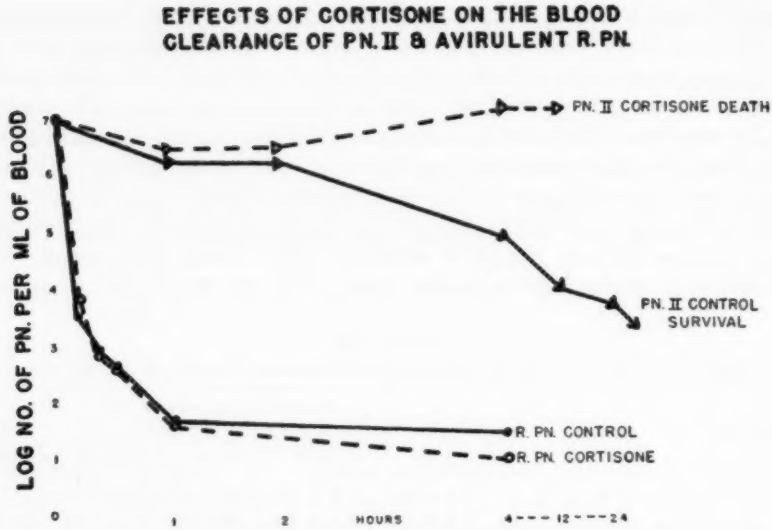
EFFECTS OF CORTISONE ON THE BLOOD CLEARANCE OF PN. II & AVIRULENT R. PN.
LOG NO. OF PN. PER ML OF BLOOD
PN. II CORTISONE DEATH
PN. II CONTROL SURVIVAL
R. PN. CONTROL
R. PN. CORTISONE
HOURS

FIG. 6. After data of Table VIII. Medians of Table VII demonstrating the course of infection with encapsulated Type II pneumococcus are shown for comparison.

TABLE IX

The Effect of Cortisone on the Blood Clearance of Pneumococcus Type II Following Passive Immunization

TIME AFTER INJECTION	CORTISONE-TREATED*		CONTROL	
	Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4
	No. of organisms per ml. of blood		No. of organisms per ml. of blood	
2 min.	320,000	110,000	720,000	72,000
10 min.	360	720	160	720
20 min.	30	6	48	70
30 min.	18	38	18	184
1 hr.	2	14	12	18
2 hrs.	2	270	0	2
4 hrs.	2	2	4	16
6 hrs.	0	0	0	0
12 hrs.	2	4	2	0
24 hrs.	382	556	12	0
48 hrs.	2	44	0	54

* Received 30, 20 and 10 mg of cortisone 72, 48 and 24 hrs. prior to intravenous infection with 1×10^9 Pn II, and 10 mg of cortisone for 1 day thereafter. In both groups of animals, 4 ml. of a pooled antipneumococcus Type II rabbit serum containing approximately .4 mg. antibody N per ml were injected intravenously 5 minutes before challenge.

The failure of cortisone to alter the blood clearance of avirulent pneumococci and the clearance of encapsulated pneumococci in passively immunized

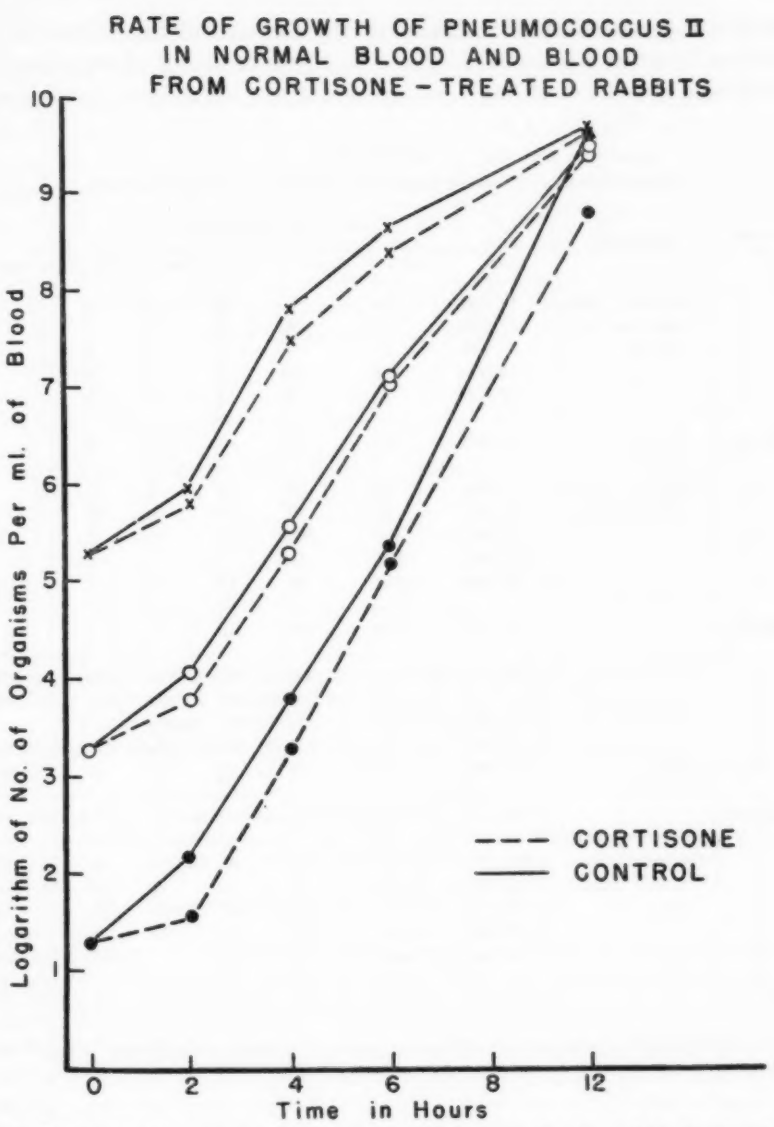

FIG. 7. Two rabbits were treated with cortisone at consecutive doses of 30, 20, 10 and 10 mg per day for 4 days. Five milliliter portions of blood obtained from these treated rabbits and two controls were seeded with approximately ten, one thousand and one hundred thousand organisms and incubated at 37° C. At varying intervals, 0.5 ml. of the blood culture was removed and the number of organisms was determined by the pour-plate technique. The averages are plotted in the figure.

animals suggested that the enhancement by cortisone of the bacteremia in virulent infection was most likely due to a greater ability of organisms to proliferate in the animal body. An experiment was undertaken to determine

TABLE X

Effect of Cortisone on Immune Response to Pneumococcus Type II Infection

RABBIT NO.	TREATMENT	COLONY COUNT PER ML. OF BLOOD							
		2 min.	15 min.	30 min.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.
1	Cortisone and previous infection	15,000	24	8	12	4	4	4	
2		13,000	32	24	16	4	8	0	
3		794	22	4	0	0	0		
4		70,000	124	20	4	0	2		
5		25,000	36	8	2	0	4		6
Median		15,000	32	8	4	0	4		
6	Control; previous infection	5,500	32	6	6	8	32	0	
7		33,000	42	10	60	6	50	4	
8		33,000	580	104	16	18	6		
9		610,000	194	18	6	4	4		
10		56,000	260	56	12	0	2		0
Median		33,000	194	18	12	6			
11	Previously treated with cortisone. No previous infection	2,000,000	1,600,000	1,200,000	440,000	90,000	1,400	1,100	8,800
12		2,000,000	1,100,000	810,000	230,000	30,000	2,400	378	606
13		5,000,000	2,800,000	1,700,000	150,000	Dead[1]			
14		18,000,000	7,200,000	2,200,000	320,000	360,000	64,000	1,100	2,200
15		760,000	174	34	10	2	0	30	602
Median		2,000,000	1,600,000	1,200,000	230,000	60,000	1,900	1,100	2,200
16	Control; no previous infection	2,600,000	1,200,000	380,000	43,000	13,000	56	428	1,100
17		1,900,000	7,300	50	4	2	10	68	
18		4,400,000	1,100,000	470,000	140,000	27,000	634	422	72
19		4,500,000	19,000	9,000	620	102	2		Dead
20		5,300,000	1,100,000	650,000	620,000		3,000	418	1,400
Median		3,500,000	1,100,000	380,000	43,000	13,000	56	422	1,100

[1] Death followed cardiac puncture which was performed because of inability to obtain blood from ear.

the rate of growth of organisms in blood removed from animals extensively treated with cortisone. The results are presented graphically in figure 7. As shown there, the rate of growth was similar in the samples of blood from both the control and cortisone-treated rabbits.

C. The effect of cortisone on the immune response to pneumococcal infection

In view of the inhibitory action of cortisone on circulating antibody (11, 13), a study was undertaken to determine whether treatment with cortisone during infection would suppress the immune response which ordinarily develops following infection. A series of 10 rabbits were injected intravenously with 1 billion pneumococcus type II organisms. Five were treated with successive doses of 30, 20, 10, and 10 mg. of cortisone for the first 4 days and with 5 mg. daily for the next 4 days. Beginning 24 hours after infection, all 10 rabbits

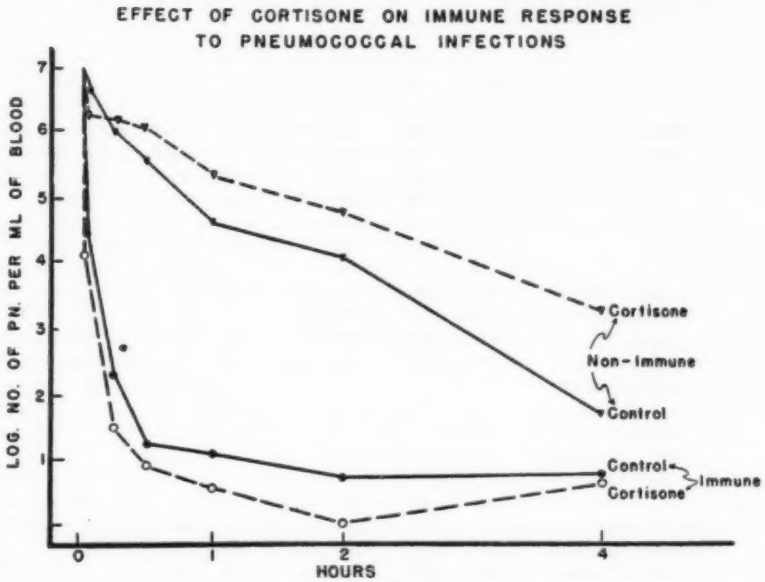

FIG. 8. After data of Table X.

received 0.2 ml. of procaine penicillin in oil containing 10,000 units per ml. intramuscularly every 12 hours for 5 days. This treatment was carried out to terminate the otherwise lethal infection ordinarily observed in the cortisone treated group. Another group of 5 normal animals received cortisone in dosages similar to the above to serve as controls. Three weeks after the first infection, all 3 groups of animals, plus a fourth group of normal rabbits, were challenged intravenously with 1 billion pneumococcus type II organisms and the rate of clearance of these organisms determined. The data are presented in Table X and figure 8. The control rabbits, which had been previously treated with cortisone but had not received a previous infection, showed a blood clearance slightly slower than the normal controls, but since the cortisone had been dis-

TABLE XI

Effect of Cortisone on the Blood Clearance of Staphylococci

RABBIT NO.	TREATMENT	COLONY COUNT (IN THOUSANDS) PER ML. OF BLOOD								
		2 min.	6 min.	9 min.	12 min.	15 min.	30min.	1 hr.	2 hrs.	24 hrs.
1	Cortisone; 30, 20 and 10 mg. repeated for 8 days prior to infection and 10 mg. thereafter; Infected with 10^9 organisms	3,900	470	100	440	56	34	9	.81	
2		9,600	680	680	130	340	300	11	2.9	
3		21,000	900	370	310	220	43	120	4.1	
Median		3,900	680	370	310	220	43	11	2.9	
5	Control. Infected with 10^9 organisms	2,100	580	58	40	11	8.7	3.3	.24	
6		15,000	700	340	160	87	8.5	2.2	.49	
7		1,700	150	81	33	43	12	.74	.39	
8		2,800	480	150	20	36	30	5.1	1.6	
Median		2,800	580	150	40	43	12	3.3	.49	
9	Cortisone; 30, 20 and 10 mg. repeated for 8 days prior to infection and 10 mg. thereafter; Infected with 10^{10} organisms	4,900	6,900	21	12	35	25	27	9.8	.95
10		34,000	780	1,000	87	71	75	22	81	1.1
11		7,200	1,900	290	110	66	190	20	2.1	.58
12		4,100	3,400	640	44	25	90	55	26	15
Median		7,200	3,400	640	87	66	90	27	26	1.1
13	Control. Infected with 10^{10} organisms	2,200	2,000	280	260	150	40	130	12	.35
14		29,000	790	470	46	89	68	49	41	.80
15		2,700	530	62	54	37	18	7.2	8.5	Dead
16		27,000	1,200	810	1.7	270	48	19	32	Dead
Median		27,000	1,200	470	54	150	48	49	32	

continued at least 2 weeks prior to infection, no progressive septicemia ensued. Both the previously infected controls and the previously infected cortisone-treated rabbits showed a rapid clearance of organisms, indicating that there had been a definite immune response to infection in both groups of animals.

II. THE EFFECT OF CORTISONE ON STAPHYLOCOCCAL BACTEREMIA AND SURVIVAL FOLLOWING STAPHYLOCOCCAL INFECTION

To investigate further the effect of cortisone on bacteremia, the action of this hormone on the blood clearance of a strain of hemolytic *Staphylococcus*

aureus was studied. The rate of removal from the blood of this organism was found to be more rapid than that with the encapsulated pneumococcus II, although less rapid than that of the avirulent strain of pneumococci. A series of 16 rabbits were divided into 2 groups. Eight of these were challenged with 1 billion staphylococci and 8 others with 10 billion organisms. One-half of each group was treated with cortisone for 8 days prior to infection and for 2 days thereafter at a dosage given in Table XI. As shown in this table and in figure 9, cortisone had no effect on the blood clearance of staphylococci.

Contrary to the deleterious effect of cortisone on survival following pneumococcal infection, it was noted that cortisone appeared to protect animals with

TABLE XII

Effect of Cortisone on the Survival of Rabbits Infected with Staphylococcus Aureus

	NO. OF RABBITS	INOCULUM	TREATMENT	SURVIVORS	DEATHS
Exp. 1	4	1 billion	Cortisone, 30, 20 and 10 mg. thereafter for 10 days prior to infection and for the following 2 days	4	0
	4		Control	3	1
Exp. 2	4	10 billion	Cortisone; 30, 20 and 10 mg. thereafter for 7 days prior to infection and for the following 2 days	4	0
	4		Control	2	2
Exp. 3	4	10 billion	Cortisone; 30, 20 and 10 mg. for 3 days prior to infection and for 2 days thereafter	4	0
	4		Control	2	2
Total....	12 cortisone-treated			12	0
	12 controls			7	5

staphylococcal infection These data are presented in Table XII. Of a total of 12 infected animals previously treated with cortisone, all survived following intravenous infection. However, 5 of 12 controls or 42 per cent died within 24 to 48 hours despite the absence of a progressive bacteremia. It was considered that cortisone might have protected by suppressing the toxic action of the hemolytic staphylococcus.

III. THE EFFECT OF CORTISONE ON THE PYROGENICITY AND LETHAL ACTION OF TYPHOID VACCINE

In various febrile diseases, cortisone or ACTH therapy has brought about a return in temperature to normal and a reduction in the toxemia, although the underlying nature of the disease has not been greatly affected (3, 14, 15).

In light of these observations, studies were undertaken to determine the effect of cortisone on the pyrogenicity and the lethal action of a typical endotoxin, such as is found in typhoid vaccine. A series of 12 rabbits were injected with 4 ml. of a formalinized typhoid vaccine containing 1 billion typhoid bacilli per ml. Six of the rabbits were treated with 30, 20, and 10 mg. of cortisone at 72, 48, and 24 hours before challenge and with 10 mg. on day of challenge. Rectal

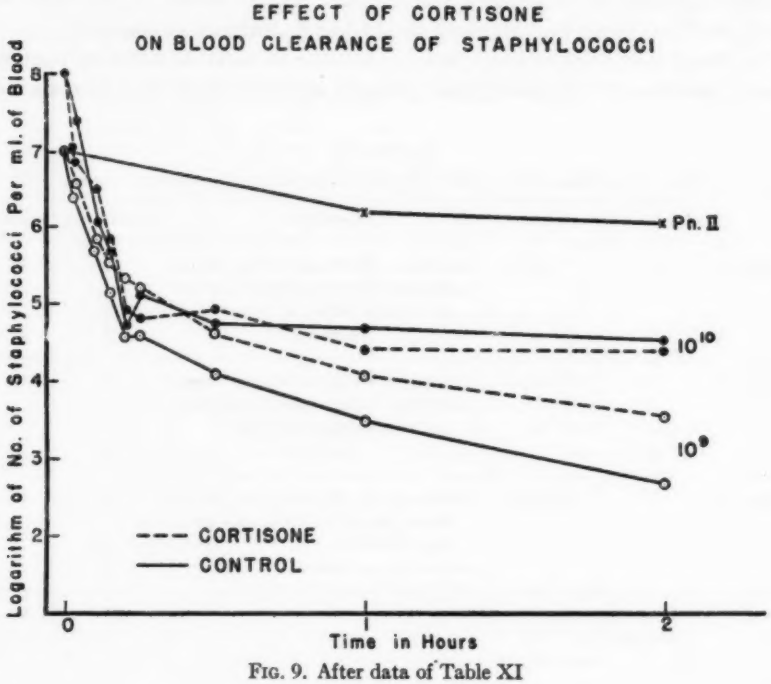

FIG. 9. After data of Table XI

temperatures were obtained immediately prior to the injection of typhoid vaccine and at 1, 2, 3, and 6 hours thereafter. The results are presented in Table XIII. As shown there, cortisone exerted an antipyretic effect on the febrile response to the vaccine. In Table XIV are presented the data on the survival of animals treated with varying doses of cortisone and receiving varying quantities of typhoid vaccine. Of a total of 26 control rabbits, 14 animals or 52 per cent died, whereas only 2 of the 26 treated animals or 7.4 per cent succumbed to the toxic action of the typhoid vaccine.

DISCUSSION

The observations recorded here indicate that treatment with cortisone may initiate a number of complex biological alterations, some of which may be

TABLE XIII

The Antipyretic Effect of Cortisone

RABBIT* NO.	TREATMENT	TEMPERATURE °C AT					
		0	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.
1	Cortisone 20, 10, and 10 mg. at 72, 48 and 24 hrs. prior to testing and 10 mg. on same day	39.8	40.2	40.8	41.5	41.2	39.7
2		40.3	40.1	40.2	40.5	40.6	Dead
3		40.0	40.0	41.3	41.5	41.5	40.4
4		40.0	39.9	40.0	40.8	40.8	39.4
5		39.7		41.4	41.8	41.9	40.3
6		40.2		40.3	40.0	39.9	40.1
Average		40.0	40.1	40.7	41.0	41.0	40.0
7	Control	40.2		41.6	42.5	42.2	Dead
8		39.3		41.3	41.7	42.5	41.1
9		39.4	40.9	41.3	41.9	42.1	40.4
10		39.5	41.2	41.7	42.2	42.1	41.9
11		39.5	40.9	41.6	42.0	42.2	41.9
12		39.4	39.9	40.5	41.7	41.4	40.9
Average		39.6	40.7	41.3	42.0	42.1	41.2

* Rabbits injected with 4 ml. of formalinized typhoid vaccine containing 1×10^9 typhoid bacilli per ml.

TABLE XIV

The Effect of Cortisone on Toxicity of Typhoid Vaccine

NUMBER OF RABBITS	QUANTITY OF VACCINE INJECTED	TREATMENT	NO. OF DEATHS	% MORTALITY
15	0.1 mg. of nitrogen by weight	Cortisone 4 mg. per day from onset of challenge	0	0
15		Control	6	40
6	2×10^9 organisms	Cortisone, 30, 20, and 10 mg. 72, 48 and 24 hrs. prior to challenge and 10 mg. per day thereafter	0	0
6		Control	4	67
6	4×10^9 organisms	Cortisone as above	2	33
6		Control	4	67
Summary				
Total 26		Cortisone	2	7.4
26		Control	14	52

beneficial to the infected host while others clearly increase the susceptibility of the host to infection. Rabbits treated with cortisone were protected against the toxic action of typhoid vaccine and survived lethal doses of a strain of

Staphylococcus aureus. Moreover, treatment with cortisone inhibited the febrile response of rabbits infected with pneumococci as well as those inoculated with heat-killed suspensions of typhoid bacilli. These effects are in agreement with the reported favorable action of this hormone on the toxemia and fever[1] of many of the acute febrile illnesses of man (3, 14, 15).

In many experimental infections, treatment with cortisone results in hypersusceptibility of the host to infection. In the present study, cortisone markedly enhanced the susceptibility of rabbits to pneumococcal infections. Aside from producing a greater mortality in the treated animals, the bacteria exhibited an increased tendency to survive and proliferate in the locally infected sites and to enter and multiply within the circulating blood.

One of the outstanding features of the local intracutaneous infection in the cortisone-treated animals was a temporary suppression of the inflammatory reaction. Subsequently, the inflammatory exudate consisted of numerous bacteria and relatively few neutrophilic leukocytes. It was apparent that the failure of leukocytes to migrate into the infected area prevented phagocytosis of the bacteria and allowed them to multiply freely within the tissues of the host. These findings are consistent with those observed in rats with experimental streptococcal pneumonia (5).

The most striking characteristic of animals treated with cortisone was a tendency to develop a fulminating bacteremia. The greater local multiplication of bacteria in the cortisone-treated rabbits probably favored a hematogenous spread of infection. However, even when cultures of encapsulated pneumococci were introduced directly into the bloodstream, the course of bacteremia was entirely different from the normal. In many of these animals following treatment with cortisone, there appeared after a short initial period of normal clearance, a steadily increasing bacteremia. Similar results have been reported in a recent communication by White and Marshall (16).

In regard to the possible mechanisms by which cortisone exerts this effect on bacteremia, it is of interest that this hormone had no influence on the *transient* bacteremia produced by the intravenous injection of avirulent pneumococci or cultures of a strain of hemolytic *Staphylococcus aureus*. These findings suggest that the abnormal course of bacteremia in cortisone-treated animals infected with virulent pneumococci was due to increased bacterial growth rather than the result of interference with the blood clearing mechanisms of the host. That the blood of the cortisone-treated animals in itself accounted for the enhancement of bacterial growth was not confirmed by *in vitro* experi-

[1] Whether the antipyretic action of cortisone is entirely beneficial may be questioned. It is conceivable that a reduction in fever might increase susceptibility to infection either by suppressing the protective mechanisms of the host or by increasing the apparent virulence of the invading organism. This latter mechanism has been shown to operate in infection in rabbits produced by some strains of Type III pneumococci. (18)

ments. In view of the inhibitory effect of cortisone on the inflammatory cellular response following injury, the possibility exists that many of the bacteria which normally leave the blood and enter the lymphatics, lymph nodes and other sites in the body (17) may now undergo a more rapid multiplication at these sites. A continuous dissemination of bacteria from these tissues into the blood could account for the apparent increased bacterial growth in the blood of the cortisone-treated rabbits.

From a consideration of the observations of the inhibitory effect of cortisone on circulating antibody levels, cortisone might well be expected to have a deleterious effect on infections. However, the effects obtained here are obviously too early to be attributed to this mechanism. Furthermore, the results of the present study indicate that treatment with cortisone during infection does not interfere with the subsequent development of acquired immunity. This finding is not surprising since the amount of antibody required for the immune response is so small that only complete suppression of antibody formation by cortisone would suffice to obtain inhibition of this phenomenon.

SUMMARY

1) Treatment with cortisone increased the susceptibility of rabbits to pneumococcal infection as manifested by (a) a greater mortality, (b) increased bacterial growth in the locally infected sites and (c) an enhanced bacteremia.

2) Evidence was obtained which suggested that the increased bacterial growth resulted from interference with the migration of phagocytic cells to the infected sites.

3) Treatment with this hormone did not appear to affect the blood clearing mechanisms of the host nor did it interfere with the development of an immune response following infection.

4) Cortisone protected animals against the toxic effects of typhoid vaccine and cultures of a strain of *Staphylococcus aureus*.

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A HEMORRHAGIC DISORDER OCCURRING IN PATIENTS WITH CYANOTIC CONGENITAL HEART DISEASE*

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Serious and even fatal postoperative hemorrhage has occurred in a small group of patients with cyanotic congenital heart disease, chiefly in adults and adolescents. In over 1100 patients operated on for pulmonic stenosis† by Blalock and his associates, fatal postoperative hemorrhage has been encountered in 18. Bahnson and Ziegler reported the clinical findings in the first seven of these fatalities (1). Subsequently there have been 11 more such cases in the pulmonic stenosis series. In addition one patient in 72 undergoing surgical treatment for transposition of the major vessels without pulmonic stenosis died of unexplained postoperative hemorrhage.

In five of these patients bleeding from the anastomotic site was proved by re-exploration or at postmortem examination. In addition one patient received dicumarol postoperatively and one heparin although in the latter case hemorrhage had apparently preceded the administration of the drug. A detailed consideration of the cause of hemorrhage in most of these cases will not be presented since few hematological studies were performed. Nevertheless, a hemorrhagic diathesis seemed to be present in most of these patients, and indeed, re-exploration in two cases revealed generalized bleeding from the chest wall and mediastinum, but no local bleeding point. Typically little or no excessive bleeding was encountered at the time of operation, but within several hours thereafter severe and uncontrollable hemorrhage began. In addition troublesome but non-fatal hemorrhage is encountered occasionally in adults undergoing operation for cyanotic congenital heart disease (2).

In a study of 31 cases of cyanotic congenital heart disease‡ a surprisingly

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† The patients underwent operation for pulmonic stenosis. However, there were usually other associated cardiac abnormalities. In the majority of cases the pulmonic stenosis was part of a Tetralogy of Fallot. In others pulmonic stenosis was associated with tricuspid atresia, transposition of the major vessels, single ventricle, or truncus arteriosus.

‡ With one exception these patients had secondary polycythemia. Although cyanosis can occur in the absence of polycythemia, the term "cyanotic congenital heart disease" is used throughout this paper in a general sense to describe patients with both cyanosis and polycythemia. In addition two patients with pure pulmonic stenosis were studied. In neither was there clinical or laboratory evidence of cyanosis.

high incidence of potential hemorrhagic diathesis was found. Three of these patients had had serious postoperative hemorrhage, and two of the three died of uncontrollable diffuse bleeding from the wound. The remaining 28 patients were studied pre-operatively and displayed no evidence of abnormal bleeding at that time, but several gave a history of excessive bleeding following minor surgical procedures. Marked impairment of clot retraction due to a combination of thrombocytopenia and low blood fibrinogen in the presence of a high hematocrit was the most striking finding.

I. METHODS

Increased blood viscosity due to the marked secondary polycythemia in cyanotic congenital heart disease may cause considerable technical difficulty in obtaining satisfactory specimens of blood by venipuncture. This was overcome by the use of silicone†-treated syringes. When blood specimen tubes containing *dry* oxalate crystals are employed, the relatively small proportion of plasma present may be insufficient to dissolve the dry oxalate crystals rapidly or completely enough to prevent coagulation. Therefore, the dry oxalate was moistened with one very small drop of 0.85% sodium chloride solution, and the clumps of oxalate crystals broken up with an applicator stick. The very small amount of saline used was insufficient to alter the hematocrit reading of the blood significantly.

Platelet counts were performed in duplicate by a direct method using Rees-Ecker diluent. Normal concentrations are 200,000 to 400,000 per cmm. The presence of a large number of red cells did not interfere with the accuracy of the platelet counts. In a number of cases the counts were confirmed by the phase microscopy technique of Brecher and Cronkite (3). In this method the red cells are hemolysed by the diluting fluid.

The *fibrinogen concentration* of plasma was determined in duplicate by the method of Ratnoff and Menzie (4). Fifty-seven of 61 normal individuals had fibrinogen concentrations ranging between 208 and 366 mg. per 100 ml. of plasma with this method (5). *Whole blood* fibrinogen values were calculated using the hematocrit and the plasma fibrinogen concentration.

Clot retraction was measured in a semi-quantitative manner by a modification of the method of Lucia and his associates (6). The serum expressed from the clot during retraction was measured. This was termed the "residual serum." The total quantity of serum in the blood specimen was calculated from the hematocrit. The amount of serum liberated from the clot during retraction ("residual serum") was expressed as a percentage of the total serum in the specimen. This was termed the "% potential residual serum." In normal blood over 80% of the serum was liberated from the clot during retraction.

Silicone-treated tubes were employed to overcome the technical difficulty of adherence of the clot to plain glass surfaces. A very small amount of thromboplastin was added to the tubes so that clotting occurred promptly. At the end of one hour the clot was removed by means of a paper-clip which had been suspended in the blood before clotting occurred. Each test was performed in duplicate at 37° C. Since both red cells and serum were present outside of the clot, the former were separated by centrifugation and the residual serum measured.

The *clotting time* determinations were carried out by a three tube method. One ml. of blood was placed in each of three Pyrex test tubes (13 × 100 mm.) and the test carried out at 37° C. Only the clotting time in the third tube was recorded for the sake of brevity. With this method normal individuals have clotting times as long as 30 minutes. The *pro-*

† Dri-film 9987, General Electric Company.

thrombin times were determined in duplicate by a one-stage method employing rabbit brain thromboplastin. The normal range is from 16 to 21 seconds by this method.

Bleeding times were performed routinely by the Duke method, but in some instances this was checked by the method of Ivy and his associates (7). The normal bleeding time by both methods varied from two to five minutes. The Rumpel-Leede *tourniquet test* was used as a measure of capillary fragility.

The effective *proteolytic (fibrinolytic) activity* of plasma was measured by the time required for complete lysis of the plasma clot (8). The test was carried out in duplicate at 37° C. Lysis of the sterile recalcified clot obtained from normal oxalated plasma requires more than 48 hours. When complete lysis of the clot occurred in less than 48 hours, the material was cultured. If the contents were not sterile, the test was not considered valid.

II. SERIOUS POSTOPERATIVE HEMORRHAGE

Case reports

Patient 1. (E. H., JHH 468751). This 25 year old white male gave a history of cyanosis from birth and life-long dyspnea on exertion. The clinical impression was that of pulmonic stenosis either associated with a widely patent foramen ovale or as part of a Tetralogy of Fallot. There was no history of abnormal bleeding. The spleen was not palpable. The pre-operative hematocrit was 76 and the prothrombin time 24 seconds. At the time of pulmonary valvulotomy no excessive bleeding was encountered and hemostasis seemed complete on closure of the chest wall. However, within several hours intrathoracic hemorrhage began. Twenty-six hundred cc. of blood drained from the chest catheter during the first 24 hours, and the patient was given 3000 cc. of citrated whole blood. The following morning he continued to bleed and despite the administration of 3000 cc. of citrated whole blood went into severe shock. A large hematoma of the chest wall developed at the site of the incision. On re-exploration generalized bleeding from the chest wall and mediastinum was encountered but no local bleeding point. At least part of the blood in the chest cavity was clotted. The patient died seven hours after the re-exploration despite the further administration of 1500 cc. of citrated whole blood and 6000 cc. by direct transfusion. Autopsy revealed evidence of marked bleeding from the chest wall. The anatomical diagnosis was Tetralogy of Fallot with pulmonary valvular stenosis.

Patient 2. (S. F., JHH 489977). This 21 year old white male gave a history of cyanosis from birth and life-long dyspnea on moderate exertion. A clinical diagnosis of Tetralogy of Fallot with pulmonic valvular stenosis was made. On several occasions the patient had bled excessively after dental extractions. The spleen was not palpable. The pre-operative hematocrit was 83 and the prothrombin time 23 seconds. At the time of pulmonary valvulotomy no abnormal bleeding was noted. However, several hours later severe hemorrhage began. The patient received a total of 26 transfusions of whole blood, six by a direct method, and 1000 cc. of plasma. Despite this, bleeding did not stop and shock could not be controlled. The patient died seven hours after termination of the operation. Autopsy revealed no evidence of a local bleeding point. The clinical diagnosis was confirmed by the postmortem examination.

Patient 3. (J. S., JHH 587627). This 24 year old white male gave a history of cyanosis since birth, life-long dyspnea on mild exertion, and ankle edema since the age of 17. A clinical diagnosis of Tetralogy of Fallot was made. There had been repeated episodes of epistaxis and bleeding gums. The spleen was palpable 4 cm. below the left costal margin, and the liver 6 cm. below the right costal margin. The pre-operative hematocrit was 85 and the prothrombin time 20 seconds. An anastomosis between the left subclavian and pulmonary

TABLE I

Hemorrhagic Diathesis in Congenital Heart Disease

PATIENT	AGE (yrs.)	DIAGNOSIS	PLATELETS *per cmm.*	HEMATOCRIT %	FIBRINOGEN Plasma *mg.%*	FIBRINOGEN Blood *mg.%*	CLOT RETRACTION %	PROTHROMBIN TIME *sec.*	CLOTTING TIME *min.*	COURSE AND REMARKS
				A. Cyanotic Congenital Heart Disease						
1	25	Tetra. with P.V.S.	64,000	55*	91	41	62	27	58	Fatal postoperative hemorrhage
2	21	Tetra. with P.V.S.	90,000	32*	99	67	23	24	58	Fatal postoperative hemorrhage
3	24	Tetralogy	44,000	63*	353	131	65	22	33	Serious postoperative hemorrhage
4	23	Tetralogy	48,000	80	168	34	6	28	33	Pre-operative phlebotomies and splenectomy
5	1 mo.	Tetralogy	52,000	61	—	—	—	—	—	No operation
6	2	Tetralogy	94,000	66	222	75	50	21	16	Operation without hemorrhage
7	2½	Tetralogy	86,000	62	—	—	—	—	—	Operation without hemorrhage
8	2½	Tetralogy with pulmonary atresia	122,000	79	—	—	27	25	25	Died at operation. Cardiac arrest. No abnormal bleeding
9	3	Tetralogy	46,000	77	176	40	30	25	12	Operation without hemorrhage
10	3	Tetralogy	73,000	63	143	53	21	26	15	Operation without hemorrhage
11	4	Tetralogy	166,000	63	—	—	—	19	14	Operation without hemorrhage
12	4½	Tetralogy	94,000	70	—	—	—	—	—	Operation without hemorrhage
13	5½	Tetralogy	98,000	68	—	—	—	—	—	Operation without hemorrhage
14	6	Tetralogy	120,000	75	184	46	<12	27	16	Operation without hemorrhage
15	7	Tetralogy	212,000	54	236	109	83	23	27	Operation without hemorrhage
16	8	Tetralogy	182,000	67	—	—	—	—	14	Operation without hemorrhage
17	11	Tetralogy	182,000	68	271	87	76	18	21	Operation without hemorrhage
18	11	Tetralogy	176,000	73	127	34	12	22	21	Operation without hemorrhage
19	12	Tetralogy	150,000	79	211	44	32	21	50	Operation without hemorrhage
20	12	Tetralogy	344,000	48*	222	115	97	22	24	No operation
21	17	Tetra. with P.V.S.	210,000	72	238	67	65	23	27	Operation without hemorrhage
22	27	Tetralogy	136,000	83	370	63	<10	22	66	Died pre-operatively
23	36	Tetralogy	126,000	81	339	64	29	19	18	No operation

23	36	Tetralogy	126,000	81	339	64	29	19	18	No operation
24	1 mo.	Trans. with P.S.	171,000	46	—	—	—	—	—	No operation
25	2½	Transposition	64,000	68	—	—	—	—	—	Operation without hemorrhage
26	2½	Trans. with P.S.	72,000	83	177	30	16	22	65	Operation without hemorrhage
27	5½	Transposition	22,000	86	—	—	—	—	—	No operation
28	2½	Tricuspid atresia	38,000	62	—	—	—	—	—	Operation without hemorrhage
29	30	Taussig-Bing	62,000	71	343	99	52	23	32	No operation
30	13	Ebstein's	72,000	72	416	116	40	23	42	No operation
31	18	Ebstein's	66,000	80	168	34	9	28	30	No operation
		B. Acyanotic Congenital Heart Disease								
32	12	Pure pulm. stenosis	127,000	42	134	78	53	24	32	Operation without hemorrhage
33	22	Pure pulm. stenosis	130,000	52	296	142	90	25	25	Operation without hemorrhage
34	3½	I.V.S.D.	120,000	32	—	—	—	—	—	No operation
35	7	I.V.S.D.	340,000	43	—	—	—	—	—	No operation
36	6	I.A.S.D.	181,000	40	—	—	—	—	—	No operation
37	3	Patent ductus art.	258,000	44	—	—	—	—	—	Operation without hemorrhage
38	6	Patent ductus art.	200,000	36	—	—	—	—	—	Operation without hemorrhage
39	7	Patent ductus art.	150,000	40	—	—	—	—	—	Operation without hemorrhage
		C. Polycythemia Vera								
40	73	Polycythemia vera	560,000	57	267	115	90	20	17	Phlebotomies. No P^{32} or x-ray therapy
41	52	Polycythemia vera	300,000	69	282	87	84	17	21	Previous P^{32}
42	55	Polycythemia vera	186,000	55	222	100	81	16	27	Previous P^{32}
43	43	Polycythemia vera	100,000	72	273	76	49	21	33	Previous total body radiation

* These relatively low hematocrit values were due to hemorrhage.

KEY TO DIAGNOSES: Tetralogy = Tetralogy of Fallot with presumed or proved infundibular stenosis. Tetra. with P.V.S. = Tetralogy of Fallot with pulmonic valvular stenosis. Transposition = Transposition of the major vessels. Transposition with P.S. = Transposition of the major vessels with pulmonic stenosis. I.V.S.D. = Interventricular septal defect. I.A.S.D. = Interauricular septal defect. Patent Ductus Art. = Patent ductus arteriosus.

The cases of polycythemia vera were included merely for convenient comparison of the hematological data with that in secondary polycythemia associated with cyanotic congenital heart disease.

arteries was carried out. The patient appeared to tolerate the procedure well, and no abnormal bleeding was noted during the operation. Five hours later massive intrathoracic hemorrhage manifested by drainage of copious amounts of blood through the chest catheter began. The patient's condition remained critical for a period of 24 hours during which time 6500 cc. of blood drained through the chest catheter, and he was given 20 transfusions, three by a direct method. Subsequently the patient developed a large clotted hemothorax which was treated with streptokinase and streptodornase with satisfactory drainage of liquified material. Nevertheless, he continued to run a septic course due to a complicating pyothorax unresponsive to chemotherapy, antibiotics, and surgical drainage. The patient succumbed on the 34th postoperative day. The possibility of subacute bacterial endarteritis had been considered. Permission for autopsy was not obtained.

These patients had not had complete hematological studies pre-operatively, but all three had marked thrombocytopenia and impaired clot retraction at the time of study. Detailed results are shown in Table I. Patients 1 and 2 were studied during the bleeding episodes. The preoperative hematocrit readings had been 76 and 83 respectively. The plasma fibrinogen concentrations were severely reduced during the fatal hemorrhagic episodes. Patient 3 was not studied until three days after operation and after the hemorrhage had stopped. The pre-operative hematocrit was 85. The plasma fibrinogen was normal at the time of study.

Bleeding time determinations were performed on patients 2 and 3 and were normal in both cases. There was no evidence of excessive fibrinolysis in any of the three patients. Thorough investigation concerning all three patients revealed no evidence that transfusion reactions or any medications were responsible for the hemorrhagic disorder.

III. STUDY OF A GROUP OF PATIENTS WITH CONGENITAL HEART DISEASE

In a study of 36 other cases of congenital heart disease a surprisingly high incidence of potential hemorrhagic diathesis was found, particularly in the cyanotic group. Table I gives a summary of the findings in these cases.

History of abnormal bleeding: None of these patients displayed evidence of abnormal bleeding at the time of study. However, several gave a history of abnormal bleeding of some degree. Patients 30 and 32 had had excessive hemorrhage after dental extractions. Patient 32 also experienced excessive bleeding after tonsillectomy. Patient 23 had had frequent troublesome epistaxes. Just prior to the time of study patient 20 had a severe hemoptysis. There was no history of abnormal bleeding in the other patients.

Platelet concentrations: In 15 of the 36 patients the platelet counts were below 100,000 per cmm. and in four of these below 50,000. There was no correlation between age and the platelet concentrations (Table I). Likewise, there was no distinct correlation between the hematocrit and platelet values.

Fibrinogen concentrations: Eight of 19 patients studied had *plasma* fibrinogen concentrations below 200 mg. per 100 ml. of plasma (Table I). None of the patients studied had values below 90 mg. per cent. In otherwise normal individuals reduction of the fibrinogen concentration of this degree does not cause abnormal bleeding. If, however, the fibrinogen concentrations are calculated on the basis of the amount available for clot formation in whole blood, the situation appears different. Because of the high hematocrit values the *whole blood* fibrinogen concentrations were frequently far below normal. The

calculated lower limit of blood fibrinogen concentration in the vast majority of normal subjects would be about 100 mg. per 100 ml. of blood. The values in 15 of 19 cases were less than 100 mg. per cent, and in seven of these less than 50 mg. per cent (Table I).

Clot retraction: In only three (# 15, 20 and 33) of 20 patients studied was clot retraction within normal limits. In these three the platelets were normal or only moderately reduced, the hematocrit values less than 55, and the blood fibrinogen concentrations greater than 100 mg. per cent. In patient 17 clot retraction was only slightly impaired. Twelve patients had markedly impaired clot retraction such that less than 50% of the available serum was expressed from the clot during retraction.

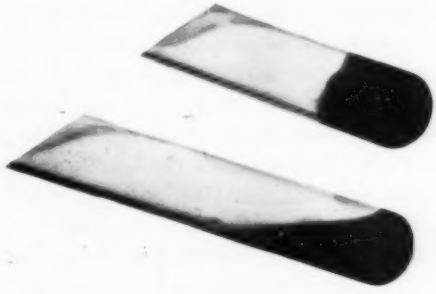

FIGURE 1. Appearance of the blood clots of patient 22. The appearance of the clots of patients 2, 4, and 26 was identical. During the clotting time determination the lower tube had been tilted every minute. The upper tube was undisturbed. It can be readily seen that the minimal agitation produced by tilting the lower tube prevented the formation of a solid clot such as formed in the undisturbed upper tube. In patients 2, 4, and 26 this phenomenon was associated with marked reduction in the blood fibrinogen, but fibrinolytic activity of the plasma was normal. In patient 22 there was excessive fibrinolytic activity of the plasma.

Clotting times: The clotting times were moderately prolonged in a few patients. In several an unusual phenomenon occurred. The blood in the first of a series of three tubes which was tilted every minute to determine the time of clotting, never formed a solid clot. The blood in the second and third tubes clotted solidly. In other words the very minimal agitation of tilting the first tube every minute prevented the formation of a solid clot (Figure 1). In three patients (# 2, 4, and 26) this phenomenon was associated with low fibrinogen concentrations. Fibrinolytic activity of the plasma was normal in these three patients. However, the plasma of another patient (# 22) whose clots presented this markedly abnormal appearance showed increased fibrinolytic activity.

In three patients with prolonged clotting times a sensitive test to detect

the presence of circulating anticoagulant substances was carried out (9). Patients 1 and 2 were studied during the fatal hemorrhagic episodes. In none was there evidence of any circulating clotting inhibitor.

Prothrombin times: The plasma from 19 of 24 patients examined showed slight to moderate prolongation of the one-stage prothrombin time (Table I). However, in none of these was the prothrombin time prolonged to levels usually associated with hemorrhagic manifestations. Even during the fatal hemorrhagic episodes in patients 1 and 2 the prothrombin times were only slightly to moderately prolonged.

The prolongation of the prothrombin time in patient 4 was studied in some detail. Since the blood collected for prothrombin determinations was oxalated 10% by volume with M/10 sodium oxalate solution, the small proportion of plasma in the polycythemic blood was mixed with a relatively larger amount of oxalate than when normal blood was collected in this manner. However, it was shown that this relatively larger proportion of the anticoagulant did not account for the prolongation of the prothrombin time. Plasma was collected from patient 4 without the use of anticoagulant by means of previously described methods (10). This plasma showed the same prolongation in prothrombin time as oxalated plasma collected in the routine manner.

Special studies demonstrated that the prolongation of the prothrombin time was due to a reduction in prothrombin itself and not to a deficiency of any accessory plasma component. The hypoprothrombinemia was unresponsive to the intravenous administration of large amounts of Hykinone® and vitamin K_1 emulsion*, but the administration of 500 ml. of stored plasma reduced the prothrombin time to normal in patient 4. Although the prothrombin was moderately reduced and the fibrinogen slightly reduced in the plasma of this patient, routine liver function tests were normal. The bilirubin, alkaline phosphatase, total protein, albumin-globulin ratio, cephalin flocculation, thymol turbidity, and bromsulfalein excretion were normal. Plasma cholinesterase activity was slightly reduced to 0.59 $\triangle$ pH per hour (normal range 0.79 to 1.30)†. Patient 21 had a slight prolongation of the prothrombin time, but likewise the liver function tests were normal. The only abnormality in these tests in patient 29 was a two plus cephalin flocculation test.

Calcium concentrations: Hemorrhage due to calcium deficiency is more of a theoretical than a real possibility since the serum calcium concentration must be reduced to a level not compatible with life before the clotting time is prolonged. The serum calcium concentration was normal in the four patients studied (#4, 21, 29, 31).

Bleeding time: None of the 21 patients studied had a significantly prolonged

* Vitamin K_1 emulsion was obtained through the courtesy of Dr. R. A. Peterman, Merck & Co., Inc., Rahway, New Jersey.

† This determination was kindly performed by Dr. David Grob.

bleeding time. Cases studied included patients 2 and 3 with serious postoperative bleeding and patient 4 with a platelet count of 44,000. A bleeding time was not done on patient 27 who had a platelet count of 22,000.

Capillary fragility: None of the 18 patients studied had a positive tourniquet test.

Fibrinolytic activity of plasma: Eleven of 14 cases studied, including the three with serious postoperative hemorrhage (patients 1, 2, and 3), had normal clot lysis determinations. The sterile plasma clot of patient 19 lysed completely in less than 28 hours, this finding representing slightly increased fibrinolytic activity. This patient underwent operation without abnormal bleeding. The sterile clot from patient 30 lysed in less than 18 hours. In this patient operation has been delayed for reasons other than her hematological status.

Only the plasma of patient 22 showed strikingly increased fibrinolytic activity. The sterile plasma clot lysed completely within several hours. Although her plasma fibrinogen was normal and the calculated blood fibrinogen not extremely reduced, the blood clots were so fragile that minimal agitation prevented the formation of a solid clot as shown in Figure 1. Furthermore, because of the rapid dissolution of the clot, accurate measurement of clot retraction was not possible. This 27 year old white female had been admitted in a critical condition for possible emergency surgical intervention. Because of her serious condition operation was postponed. She developed neurological manifestations, and a diagnosis of cerebral thrombosis or abscess was made. The patient died three days after admission. Autopsy including examination of the brain failed to reveal any specific cause for her death. The relationship of the markedly accelerated fibrinolytic activity of her plasma to the terminal illness was not apparent.

IV. STUDIES CONCERNING THE ETIOLOGY OF THE THROMBOCYTOPENIA IN CONGENITAL HEART DISEASE

As was previously noted there was no correlation between the age of the patient and the degree or presence of thrombocytopenia. Platelet counts below 100,000 were limited to cases of *cyanotic* congenital heart disease with polycythemia, but there was no correlation between the degree of polycythemia and the platelet reduction. There was a modest reduction in platelet concentrations in the two patients with pure pulmonic stenosis (#32 and 33). No significant reduction in platelets was found in the cases with patent ductus arteriosus. Two patients with pulmonary arteriovenous aneurysms and hematocrit values of 62 and 53 respectively had normal platelet counts as did one adult with acquired heart disease and an hematocrit of 58.

Platelet function: The absence of prolonged bleeding times and abnormal capillary fragility even in the presence of fairly marked thrombocytopenia suggested that those platelets present functioned normally. "Prothrombin con-

sumption" tests were carried out by a modification of the method of Quick and Favre-Gilly (11). The results suggested in each instance studied that there was more than adequate utilization of prothrombin and that in this respect platelet function was also normal. Microscopic examination revealed no significant morphological abnormality of the platelets.

However, since clot retraction in specimens of whole blood was so frequently impaired, a separate study of the ability of the platelets to bring about retraction of the clot was undertaken. In order to test this properly the mass of red cells was removed from the blood of patient 30, and plasma with varying platelet concentrations prepared by a previously described method (12). Normal plasma with varying platelet concentrations was likewise prepared. The degree of clot retraction in the plasma of patient 30 at varying platelet concentrations was compared to that in normal plasma at similar platelet levels. The results demonstrated that the platelets of patient 30 were capable of producing normal clot retraction when the large mass of red cells was removed by differential centrifugation.

Absence of a circulating factor producing thrombocytopenia: Harrington and his associates have demonstrated that the plasma of patients with "idiopathic" thrombocytopenic purpura may contain a circulating substance capable of producing a fall in the platelet count of normal individuals (13). Thus, when the blood or plasma of one of their patients was administered to a subject with a normal platelet concentration, the platelet count of the latter fell dramatically. Transfusion of 750 cc. of the blood of patient 4 and 500 cc. of the blood of patient 29 into two subjects failed to depress the platelet count in either case. The recipients had carcinoma but normal platelet concentrations.

Bone marrow studies:* Considerable difficulty may be encountered in obtaining satisfactory marrow specimens by aspiration in those patients with marked polycythemia. Histological sections revealed the presence of many large blood vessels in the marrow. These vessels apparently may be easily ruptured on aspiration of marrow. In this manner the marrow may be excessively diluted with the "thick" peripheral blood.

Satisfactory aspiration specimens were obtained from patients 4, 9, 29, 31 and 32. In those portions of the smears unaffected by dilution with peripheral blood, megakaryocytes were abundant but platelet production was markedly deficient or not seen. The only histological sections of marrow obtained from patients with platelet counts of less than 100,000 per cmm. were postmortem specimens from patients 1 and 2. In the first case megakaryocytes were abundant, and in patient 2 there appeared to be no significant deviation from the number of megakaryocytes present in normal marrow.

* Bone marrow aspirations on patients with cyanotic congenital heart disease should be done with caution. The cortical bone appears to be thin and the inner table of bone may be readily penetrated if proper precautions are not taken.

Spleen size: Only in patients 3 and 32 were the spleens clinically palpable, and in neither of these two was the enlargement great. Pathological examination of the spleens of patients 1, 2, and 4 revealed some engorgement but nothing histologically significant.

V. THERAPEUTIC CONSIDERATIONS

Experience is too limited to suggest any definitely conclusive therapeutic regimen. Fortunately, serious postoperative bleeding due to a hemorrhagic diathesis does not appear to be a frequent problem in young children, who make up the vast majority of patients undergoing cardiac surgery. In general

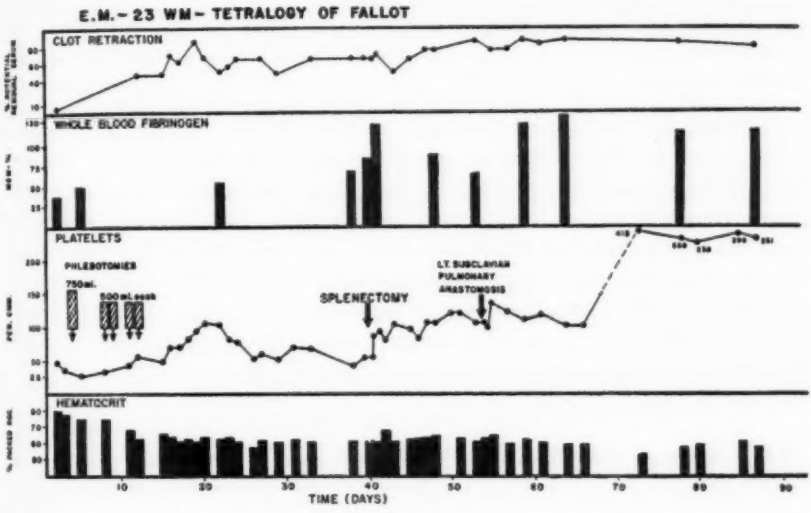

FIG. 2

even those young children with marked defects in their hematologic al test underwent operation without hemorrhage (Table I). Patient 4, an adult, will be discussed in detail because of some interesting therapeutic implications:

E. M. (JHH 591074) was a 23 year old white male who gave a history of cyanosis from birth and life-long dyspnea on exertion. He had had moderately excessive bleeding following dental extractions on one occasion. A clinical diagnosis of Tetralogy of Fallot was made, and the patient was admitted for further verifying studies. The spleen was not palpable. When first seen he bled for a period of five days from a small incision in the antecubital fossa for angiocardiography.

This patient's blood clots had the appearance shown in Figure 1, due to extremely low blood fibrinogen. Fibrinolytic activity was normal. Initially the platelet count was 48,000 and the hematocrit 80. Other details of his studies are shown in Table I and Figure 2. Repeated bleeding time determinations and tourniquet tests were normal. The marrow aspira-

tion specimens showed an abundance of megakaryocytes with markedly deficient platelet production. The Coombs test was negative.

The patient's course is shown in Figure 2. Repeated phlebotomies with equal replacement of plasma for the amount of blood withdrawn, resulted in a transient elevation of platelets to about 100,000 per cmm., a slight rise in the blood fibrinogen concentration, and improved clot retraction, the latter two chiefly at the expense of the lowered hematocrit. The blood clots became firm and no longer had the appearance shown in Figure 1. The patient tolerated the phlebotomies well. Even though the reduction in the hematocrit persisted, the platelet concentration fell to original values. Splenectomy was performed four weeks after the phlebotomies in an effort to elevate the platelet count. The operation was accompanied by moderate but readily controlled skin bleeding at the time of closure. Two weeks later and after a modest but sustained rise in platelets to about 120,000 per cmm., a left subclavian pulmonary anastomosis was performed uneventfully. Following this the platelet and fibrinogen concentrations rose to normal and have remained there for a period of observation of 18 days. It seems almost certain that this patient would have bled seriously had he been operated on when first seen.

Phlebotomies were also performed on patient 29, who was not a candidate for surgery. His hematocrit was reduced from an initial value of 76 to 60 and kept there for about three months. No striking or permanent rise in the platelet concentration was produced. On two occasions his platelets rose temporarily from the neighborhood of 60,000 to 135,000 per cmm. One of these was nine days after multiple dental extractions which he tolerated without hemorrhage, and the elevation in platelets persisted for a period of 17 days. Patient 20 was studied 13 days after a severe hemoptysis had reduced the hematocrit from 58 to 48, and the platelet count had risen from 145,000 to 344,000.

VI. CHANGES FOLLOWING CARDIOVASCULAR SURGERY

Following many surgical procedures there may be temporary changes in the platelet concentration (14–17). There may be mild depression during the first few days, followed by a rise beginning around the 6th day and reaching a maximum on the tenth to fourteenth days. This elevation may persist for several weeks. These non-specific postoperative changes make it difficult to evaluate the effect of cardiac surgery on the platelet count since most of the patients leave Baltimore about one month after the operation. However, certain observations make it seem likely that successful cardiovascular surgery corrects the platelet deficiency in a specific manner. Splenectomy in patient 4 resulted in only a modest rise in platelets, whereas the subclavian-pulmonary anastomosis produced a clear-cut rise to normal values which has been maintained for at least 18 days (Figure 2). The platelet count in patient 9 rose to normal values six days after operation and remained there during the 23 days of further hospitalization. Sixty-six days after operation her platelet count was 206,000*. Most patients showed a rise in the platelet count postoperatively.

* The author is indebted to Dr. Theodore R. Waugh, McGill University, Montreal for the follow-up examination.

However, further follow-up will be necessary to determine the permanency of this elevation of platelets.

The *plasma* fibrinogen rose to definitely normal values in patients 4, 9 and 10 following cardiac surgery. In only a few instances was clot retraction studied postoperatively. In patients 9 and 10 clot retraction became normal concommitant with postoperative reduction of the hematocrit and elevation in the concentrations of platelets and fibrinogen. In patient 4 the improvement in clot retraction brought about by phlebotomies and splenectomy continued after cardiac surgery.

Postoperative prothrombin times were determined in a few patients. Although there was a postoperative slight reduction in the prolonged prothrombin times of patients 4, 10, and 32, these values did not return completely to normal within a month of postoperative observation. In patient 9 the prothrombin time was reduced from 25 seconds to 21 seconds, a value at the upper limit of normal.

VII. HEMATOLOGICAL DATA IN POLYCYTHEMIA VERA

Four cases of polycythemia vera were studied (patients 40–43, Table I). Three had previous treatment with ionizing radiation and one with phlebotomies alone. The hematocrits were variously affected by the previous therapy giving a range from 55 to 72. The excessively high platelet counts present in untreated polycythemia vera had been reduced by ionizing radiation to normal values in patients 41 and 42 and to a subnormal level of 100,000 in patient 43. In each instance the plasma fibrinogen concentrations were normal although in patients 41 and 43 the *blood* fibrinogen was slightly below 100 mg. per cent due to the high hematocrit values.

It has been generally accepted that clot retraction in polycythemia vera is impaired despite the presence of a superabundance of platelets. This has been attributed to the presence of the high hematocrit (18). The impaired clot retraction in many instances may be more apparent than real. During the retraction of the clot from a patient with polycythemia vera a large amount of red cells may be expressed from the clot. This may give the gross appearance of a weak clot which has fallen apart and failed to retract well. When, however, a paper-clip was placed into the tube containing the blood, a moderately firm to firm clot could be withdrawn with the paper-clip. The liberation of many red cells from the retracted clot in polycythemia vera may be just an expression of efficient clot retraction in the presence of a large mass of red cells.

In each of the cases of polycythemia vera with normal or elevated platelet counts (patients 40–42) clot retraction as measured by the residual serum was normal. However, patient 43 with low platelets due to previous total body radiation had impaired clot retraction. In this limited group of patients with polycythemia vera clot retraction was normal, providing the platelet counts were normal or elevated.

DISCUSSION

Markedly impaired clot retraction was the most striking laboratory finding in the hemorrhagic diathesis of cyanotic congenital heart disease. There is good evidence to suggest that clot retraction serves a vital function as the final stage of physiological hemostasis *in vivo* in sealing the cut ends of blood vessels. Clot retraction in turn is influenced by a combination of factors. Sufficient platelets and fibrin are essential for clot retraction. In addition when the mass of red cells is large, the physical forces required to produce retraction may be much greater. Favre-Gilly and his associates have attributed the impaired clot retraction in this disease solely to the high hematocrit values (19). That this was not always necessarily so can be seen from the control studies on cases of polycythemia vera. With hematocrit values as high as 69 clot retraction was still within normal limits in polycythemia vera providing adequate platelet and fibrinogen concentrations were present. On the other hand congenital cardiac patients with hematocrit values below 69 had impaired clot retraction unless the platelet and fibrinogen concentrations were adequate (Table I). In non-polycythemic individuals platelet counts in the neighborhood of 75,000 to 100,000 per cmm. of blood are not considered to be critical in relation to the possibility of hemorrhage. However, in the presence of high hematocrit and low fibrinogen values such platelet counts may become clinically significant.

The fibrinogen concentrations deserve special comment. Foster and Whipple long ago emphasized the importance of calculating the amount of fibrinogen present in *whole blood* as that amount functionally concerned with hemostasis (20). In non-polycythemic individuals the determination of the *plasma* concentration may suffice for evaluating the adequacy of fibrinogen. However, in polycythemia the *blood* fibrinogen concentration gives a more accurate picture of the amount of fibrin available for clot formation. In cyanotic congenital heart disease the *plasma* fibrinogen was frequently slightly reduced. However, because of the high hematocrit values, the amount available in whole blood for fibrin formation was frequently far below normal. In some instances the concentration of the blood fibrinogen was so low that the clot fell apart with minimal agitation.

The concept that impaired clot retraction is the chief defect in the hemorrhagic diathesis of cyanotic congenital heart disease is quite compatible with the clinical course seen in those patients with severe bleeding episodes. Spontaneous bleeding was unusual as would be expected in the presence of normal bleeding times, capillary fragility, and relatively normal coagulation. In patients 1, 2, and 3 as well as most of the previously encountered fatal cases there was a delay period after the operation before the onset of serious bleeding. It is quite possible that vascular factors concerned with hemostasis may be sufficient at the time of operation to prevent excessive bleeding. However,

later adequate clot retraction to seal the cut ends of vessels may become essential for maintaining hemostasis. The markedly impaired clot retraction in cyanotic congenital cardiacs may then be unable to maintain effective hemostasis.

Bahnson and Ziegler have called attention to the slight prolongation of the prothrombin time in congenital heart disease (1). Favre-Gilly and his associates incriminated this as the chief cause of the hemorrhagic diathesis (19). These workers reported markedly prolonged prothrombin times, some exceeding several minutes, in a high proportion of the cases studied. However, in the present study only slight to moderate prolongation of the prothrombin times was noted, even in patients 1 and 2 studied during their fatal hemorrhagic episodes. Furthermore, these prothrombin times were significantly lower than those which may cause hemorrhage. Among the fatalities due to hemorrhage prior to this study four patients had prothrombin time determinations during the hemorrhagic episode. In only one instance was the prothrombin time longer than 26 seconds (45% of normal). In this four year old girl, previously described by Bahnson and Ziegler, the prothrombin time was 40 seconds (25% of normal).

Considerable care must be taken in obtaining satisfactory specimens for prothrombin determinations in patients with marked polycythemia because of the increased viscosity of the blood. If such care is not exercised, excessive prolongation of the prothrombin time due to technical reasons may be encountered. In the present series of patients the slight to moderate prothrombin deficiency was clearly not the cause of the hemorrhagic tendency.

The etiology of the thrombocytopenia remains obscure. Only those patients with secondary polycythemia had severe reduction in platelet concentrations. In the acyanotic group the two cases of pure pulmonic stenosis and one case of interventricular septal defect had moderate reduction of the platelet concentration. Even in the cyanotic group there was no precise correlation between the hematocrit and the platelet concentration. Furthermore, no thrombocytopenia was present in other forms of secondary polycythemia such as those associated with pulmonary arteriovenous fistula in two cases and acquired heart disease in one case. No significant alteration in platelet concentration has been noted in the polycythemia of high altitude (21). In polycythemia vera the platelet count is usually markedly elevated. Reduction of the hematocrit by repeated phlebotomies in cyanotic congenital heart disease produced only a transient rise in platelets not unlike that seen in normal individuals after hemorrhage (22).

No evidence was found that the platelets themselves were abnormal in morphology or function. Furthermore, no circulating substance capable of depressing the platelet concentration was found in the blood of cyanotic con-

genital cardiacs. Pachioli reported no increase in the proportion of megakaryocytes present in marrow aspiration smears in a group of patients with congenital heart disease (23). Most of his patients were young infants and had relatively little polycythemia. Leitner reported hypoplasia of the megakaryocytes in the marrow of one patient with cyanotic congenital heart disease (24). However, the marrow smear was hypocellular. In the present study the difficulty of obtaining cellular marrow aspiration specimens was emphasized. In satisfactory marrow specimens the megakaryocytes seemed abundant but platelet production suppressed. Splenectomy in one patient produced an immediate but modest rise in the platelet concentration which was maintained to the time of subclavian-pulmonary anastomosis two weeks later (Figure 2). Certainly there was no striking elevation of platelets after splenectomy such as is often seen in "idiopathic" thrombocytopenic purpura.

The etiology of the reduction in prothrombin and *plasma* fibrinogen concentrations likewise remains obscure. There was little evidence of abnormalities in the liver function tests. If these defects are due to abnormal liver function, the hepatic impairment must be subtle. Reduction of the hematocrit by repeated phlebotomies did not correct the prothrombin and *plasma* fibrinogen deficiencies. Additional evidence that these defects were not due to polycythemia itself was supplied by the presence of normal prothrombin and *plasma* fibrinogen concentrations in polycythemia vera.

Experience remains too limited to offer any definite therapeutic regimen for the prevention of serious postoperative hemorrhage in the cyanotic group. In this consideration the *age* of the patient seems to be a most important factor. In children fatal hemorrhage, when it occurred, was usually due to local operative causes. During the present study several children with marked abnormalities of their hematological tests had no hemorrhage at or following operation. Of the first 1131 patients in the pulmonic stenosis series, 165 were 14 years of age or over. In this age group 12 have died of postoperative hemorrhage. In only two cases was this definitely explained by bleeding solely from the suture line. This gives a gross mortality associated with postoperative bleeding of about 7% for this age group and serves to emphasize that *the problem of postoperative hemorrhage is predominantly present in adults*. On the other hand fatal postoperative hemorrhage occurred in only seven of the 966 patients under the age of 14, giving a gross mortality of 0.7% associated with postoperative bleeding. In at least three and possibly four of these fatalities it seems reasonably certain that the major cause of the postoperative hemorrhage was a defect in the suture line. No ready explanation for the fortunate finding that children in general tolerate operation well despite the potential hemorrhagic diathesis was apparent. No evidence was found to suggest that the difference between adults and children could be accounted for solely by the extent of the

collateral circulation present in the chest wall and mediastinum as judged from the operative notes.

Reduction of the hematocrit by repeated phlebotomies in selected patients seems to be a rational procedure. This was tolerated well in the several patients in whom this manuveur was tried. By this procedure the large mass of red cells was reduced and in proportion the blood fibrinogen was elevated. Both clot retraction and the mechanical strength of the clot were improved. In certain instances it may be of value to take advantage of the transient platelet elevation following phlebotomies and perform the major cardio-vascular surgery at that time. In selected patients splenectomy may be considered. In situations in which an adult with severe potential hemorrhagic diathesis requires emergency surgery, phlebotomies may be of value merely by the reduction of the large mass of red cells and elevation of blood fibrinogen. Only time and accumulated experience will definitely determine the value of these procedures.

The relationship of excessive fibrinolytic activity of plasma to clinical bleeding has not been definitely established. Mathey and his associates have described an unusual group of patients undergoing lobectomy or pneumonectomy in whom excessive fibrinolytic activity of the plasma led to uncontrollable thoracic hemorrhage at the time of the operation (25). In these patients there was incoagulability of the blood due to the absence of fibrinogen which had been lysed by the excessive proteolytic activity of the plasma. These authors conjectured that such might be the situation in the hemorrhage occurring as a result of surgery in congenital heart disease. In the present study no evidence for such a mechanism was found. Excessive bleeding was usually not encountered at the time of operation as in the patients of Mathey et al, but only several hours thereafter. No excessive fibrinolytic activity was present in the plasma of patients 1 and 2 studied during the active bleeding episodes. In the former patient solid clots were found in the chest at the time of reexploration. The autopsy protocols concerning previous postoperative fatalities due to hemorrhage were reviewed. In each instance in which specific mention of the character of the blood in the chest cavity was made, blood clots were noted. This appears to indicate that there was no total absence of fibrin and fibrinogen as in the cases of Mathey and his associates (25).

The treatment of thrombosis, a major complication of cyanotic congenital heart disease, remains a difficult problem. The temptation to use the anticoagulant drugs may be great. On the basis of the present studies their use would appear to be fraught with danger. Patients with congenital heart disease are likely to be very sensitive to the anticoagulant drugs rendering proper control of their use difficult. It has been shown that thrombocytopenic blood is more sensitive to the anticoagulant effect of heparin than blood with a normal

concentration of platelets (12). Furthermore, it is common experience that patients with prolonged prothrombin times may be very sensitive to dicumarol.

SUMMARY

1. Serious and even fatal postoperative hemorrhage has occurred in a small group of patients with cyanotic congenital heart disease, chiefly in adults and adolescents. A study of 39 patients with congenital heart disease, including two with fatal and one with serious postoperative bleeding, was undertaken.

2. A surprisingly high incidence of potential hemorrhagic diathesis was found in the cyanotic group. Marked impairment of clot retraction due to a combination of thrombocytopenia and low blood fibrinogen in the presence of a high hematocrit was the most striking finding.

3. The slight to moderate prolongation of the prothrombin time was clearly not the cause of the hemorrhagic disorder.

4. Some observations concerning the etiology of the depression in platelet, fibrinogen, and prothrombin concentrations are recorded.

5. Despite the presence of markedly impaired hematological tests in all age groups, serious postoperative hemorrhage is chiefly a problem in adults and adolescents. In children, fatal hemorrhage, when it occurred, could usually be explained by local surgical causes. In the present study children with marked impairment in their hematological tests underwent operation without hemorrhage.

6. One adult with severe impairment in hematological tests was subjected to phlebotomies and splenectomy prior to cardiac surgery. Subsequently a subclavian-pulmonary anastomosis was carried out without hemorrhage. The value of these procedures in preparing selected adults for eventual cardiac surgery must be determined by accumulated experience.

7. On the basis of these studies it would appear that the use of the anticoagulant drugs in the treatment of thrombosis in cyanotic congenital heart disease may be fraught with danger.

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A CHOLESTEROL METABOLIZING ESCHERICHIA COLI

A Preliminary Report*

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An Escherichia coli capable of metabolizing cholesterol was cultured from the duodenal drainage fluid of a patient with chronic cholecystitis. Cell free extracts and acetone-killed bacteria of this strain also possess the ability to degrade radio-carbon labeled cholesterol to carbon dioxide.

The bacillus has the morphologic and cultural characteristics of the E. coli group. It gives typical fermentation reactions and the customary findings on special media such as EMB and Kligler's iron agar.

EXPERIMENTAL

Bacterial preparations—Single colonies of the organism were picked to neopeptone meat infusion broth. After a few passages in this medium, 0.6% cholesterol was added to the media before sterilization. The organism has been maintained continuously on either beef broth or nutrient agar containing 0.6% cholesterol for more than two months.

The cell free extract was prepared as described by Hayaishi and Stanier (1). After high speed centrifugation it was passed through a Seitz filter before use.

The acetone-killed bacteria preparation was made by washing the organisms with saline from solid media into a centrifuge tube, centrifuging, washing with saline and then stirring with acetone in the cold for one-half hour. The bacteria were packed by centrifugation, the acetone removed by decanting and the bacterial precipitate lyophilized.

Substrate experiments—To determine whether the organism could use cholesterol as its only carbon source, tubes containing saline, saline plus cholesterol and meat infusion broth plus cholesterol were seeded with the organism. Bacterial counts were made by the usual method before and after a 24 hour incubation. From Table I it can be seen that the E. coli grew as well with cholesterol as its only substrate as it did with meat infusion broth plus cholesterol.

Cholesterol metabolism experiments—Weighed amounts of radiocarbon labeled cholesterol, biologically prepared from sodium acetate-1-C^{14} (2), plus 20 ml. of phosphate buffer at pH 7.4 were placed in flasks and autoclaved. The requisite bacterial preparation was added and the flasks were sealed, gassed with 95% oxygen-5% carbon dioxide and incubated at 37° C. with shaking for 24 hours. At the end of the incubation period the CO_2 was collected and counted as $BaCO_3$(3). The non-isotopic cholesterol experiments were performed in a similar manner and the cholesterol content at the end of incubation determined by the Schoenheimer-Sperry method (4).

Isotope analyses: C^{14} activity was measured with a thin window Geiger-Müller counter.

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The counting vessels were sintered steel dishes of 2.0 sq. cm. area. Where necessary, correction to infinite thickness was made from known data (5). Sufficient counts were taken to give a standard deviation of less than 5%.

TABLE I

Substrate Experiments

SUBSTRATE	NUMBER OF E. COLI PER ML. BEFORE INCUBATION	NUMBER OF E. COLI PER ML. AFTER 24 HOUR INCUBATION
0.85% saline	9,800	83,000
0.6% cholesterol in Meat Broth	9,500	48,000,000
0.6% cholesterol in 0.85% saline	16,900	69,000,000

TABLE II

*Cholesterol Degradation by E. coli Preparations**

PREPARATION	MILLIGRAMS CHOLESTEROL ADDED	RADIOACTIVITY ADDED AS CHOLESTEROL CARBON COUNTS/MINUTE†	RADIOACTIVITY RECOVERED AS CO_2 CARBON COUNTS/MINUTE‡	MILLIGRAMS CHOLESTEROL RECOVERED	PERCENTAGE CHOLESTEROL CARBON CONVERTED TO CO_2
Living E. coli	3.43	7,390	28,500		55
Living E. coli in Meat Broth§	12.62			5.90	53
Acetone-killed E. coli	3.00	786	463		9
Acetone-killed E. coli lyophilized	3.90	7,390	2,190		10
Cell free extract of E. coli	3.00	786	109		2
Cell free extract of E. coli	3.20	7,390	972		2

* Routine cultures on the last four experiments in the table were sterile at the end of the incubation period.

† C^{14} activity obtained per standard dish of added cholesterol carbon (6).

‡ C^{14} activity obtained per standard dish of CO_2 carbon recovered as $BaCO_3$ (6).

§ In this experiment the cholesterol was added as cholesteryl monosuccinate and the flask incubated for 48 hours. The 53% in the last column represents that fraction of the cholesterol unrecovered at the end of the incubation.

RESULTS

Representative results obtained by incubating E. coli organisms, acetone-killed bacteria and cell free extracts with C^{14}-labeled cholesterol as substrate are given in Table II. The ability to degrade cholesterol to carbon dioxide is most marked in the living organism but persists in even the cell free extracts. The second experiment in Table II demonstrates that this E. coli will metabolize cholesterol in the presence of other substrates. We have tested several other strains of E. coli, isolated from human feces, and none has degraded labeled cholesterol to carbon dioxide.

Specifically labeled cholesterol was not available at the time of these experiments, so precise data as to the point of enzymatic attack on the cholesterol molecule is not presented. However, Little and Bloch (2) have shown that cholesterol, biologically synthesized from acetic acid-1-C^{14}, contains 55% of

the isotope in the nucleus and 45% in the side chain. Since the cholesterol used in these experiments was prepared in this manner it seems likely that, at least in the case of the living organisms, the enzyme systems of this E. coli degrade both the $C_{19}H_{30}$ nucleus and the isooctyl side chain of cholesterol.

Fission of the steroid molecule by cultures of microorganisms of the genus Proactinomyces has been demonstrated (7, 8). Recently (9) a saprophytic mycobacterium was reported which has the ability to metabolize C^{14}-cholesterol to carbon dioxide; however, cell free extracts of the organism were inactive in this respect. The fact that killed bacteria and cell free extracts of our strain preserve their enzymatic activity in the conversion of cholesterol to carbon dioxide make this organism unique.

This E. coli demonstrates well the adaptive capacities of microorganisms in developing unusual enzyme systems, in this case presumably as a response to habitation in a biliary tract containing large amounts of cholesterol. Further, the fact that an active cholesterol degrading enzyme system can easily be isolated suggests its possible use in the control of the blood cholesterol level by enzymatic degradation of dietary cholesterol and the endogenous cholesterol passed into the gut with the bile. Experiments to determine the validity of this hypothesis are in progress.

SUMMARY

An Escherichia coli is described which contains enzyme systems capable of degrading cholesterol to carbon dioxide. The acetone-killed bacteria and cell free extracts are likewise active.

The authors are indebted to Mrs. John A. Richardson for technical assistance.

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MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

In Association with the Alpha Omega Alpha Fraternity

Monday, April 21, 1952

(The meeting was called to order and the election of officers for the coming year was held. Dr. Jonas Friedenwald was elected President and Dr. Philip Wagley, Secretary).

Dr. Grafflin: The rest of the meeting will be given over to the students under the auspices of the Alpha Omega Alpha Fraternity. All presentations will be by medical students who have had one or another interest along research lines in the course of their training here. I am going to ask Dr. Hendrix to take over the meeting from this point on.

Dr. Hendrix: At this time I would like to thank the Johns Hopkins Medical Society for turning over this meeting to the Alpha Omega Alpha Fraternity for the presentation of student papers. We would like at this time to thank all those who have shown so much interest in the individual work of these students.

The first paper this evening is by Miss Patricia McIntyre.

HYPOKALIEMIA OCCURRING DURING PAS THERAPY

Thirty-eight patients were studied who had been treated with para-aminosalicylic acid (PAS) on the medical services of The Johns Hopkins Hospital for long periods of time. Eight of these patients had a total of 12 separate episodes of hypokaliemia as determined by the serum potassium and/or electrocardiogram. Two patients were classified as "probable" hypokaliemia on the basis of well described symptoms in the absence of EKG and serum potassium determinations. Three cases were considered as "possible" hypokaliemia because of suggestive symptoms but inadequate clinical records. Twenty-five patients showed no evidence of hypokaliemia. Various types of commercially available PAS were prescribed. None of these patients received liquorice.

Considering possible etiological mechanisms, one cannot rule out some type of excessive renal excretion as no balance studies were done. However, analysis of gastro-intestinal symptoms in these patients revealed the following: 1). In the group of proved hypokaliemia seven patients, before and during eleven of the episodes, had severe vomiting and/or diarrhea. It is not possible to exclude the possibility of such symptoms in the remaining patient; 2). All of the five patients in the "probable" and "possible" groups had severe, persistent vomiting and diarrhea; 3). Only eight of the twenty-five cases with no evidence for hypokaliemia had any type of gastro-intestinal intolerance to the drug, and in seven of these eight, such symptoms were rapidly controlled by lowering the dosage or discontinuing the drug. The remaining exception had persistent vomiting on many dosages and forms of PAS.

In view of these findings it is suggested that the hypokaliemia seen in patients treated here with PAS is at least in large measure due to loss of potassium through the gastro-intestinal tract.

Dr. Hendrix: Miss McIntyre's paper will be discussed by Dr. Howard.

Dr. Howard: I am very happy to say a word about this paper and to congratulate Miss McIntyre for calling this situation to our attention. I frankly had never heard of the development of low serum potassium in patients who had been treated with PAS. I am not quite clear in my own mind as to whether Miss McIntyre thinks that the drug itself has something to do with the production of hypokaliemia over and above its capacity to produce nausea and diarrhea. I was under this impression until the Australian literature was brought to my attention showing that certain of their patients could have low serum potassium without nausea and diarrhea.

I don't think that one can rule out the possibility that other mechanisms may be involved, some of which she has mentioned. The drug might conceivably act as an exchange resin and take potassium out in the stool or it might prevent reabsorption of potassium by the renal tubules, or it might activate adrenal cortical function as has been suggested for all salicylates. Unless the new drugs render streptomycin and para-amino salicylic acid outmoded and unnecessary, certainly metabolic experiments should be carried out to determine by which if any of the suggested mechanisms the hypokaliemia is produced. For hypokaliemia is a very dangerous condition if not recognized, and can be lethal.

Dr. Fisher: Would you say whether or not serum potassiums have been determined in patients who have been given large doses of PAS intravenously?

Dr. Hendrix: Are there any other questions? Dr. Rich.

Dr. Rich: I would like to ask Miss McIntyre if there is any experimental evidence that PAS produces hypokaliemia in animals?

Miss McIntyre: In response to the first question, of Dr. Howard's about the Australian work, actually there are five groups who have described hypokaliemia occurring in patients on PAS, three English and two Australian. In reviewing the clinical abstracts of the 18 proven and possible cases reported by these groups, I found that in 9 of these patients there were some gastro-intestinal disturbances. No mention was made of the presence or absence of such symptoms in the others. Each of these articles was concerned with some other aspect of this problem and, to my knowledge, no attempt has been made to analyse it from this point of view.

Dr. Fisher, some of these patients were on intravenous PAS at one time or another. The last patient actually was on nothing but intravenous sodium PAS before she developed hypokaliemia and three of the others, just previous to this time, were put on intravenous PAS in an attempt to alleviate the same gastrointestinal symptoms I mentioned.

Dr. Rich, There has been no research done on experimental animals on any of these aspects. Anything that has been done has been done in regard to the tubercle bacillus.

Dr. Hendrix: The second paper is by Mr. William H. Miller.

THE NEURAL STRUCTURE AND FUNCTION OF AN INVERTEBRATE EYE

A fundamental problem in the study of the visual process is the recording of the electrical activity of photoreceptor cells. This has not been accomplished in the vertebrate retina. However, the eyes of some of the lower animals are being investigated because they are much less complex than vertebrate eyes. In one of these animals, *Limulus* (the horseshoe crab), the eye is especially interesting because the optic nerve is thought to arise directly in the sensory structure of the eye. The physiology of this eye has been investigated extensively by Dr. H. K. Hartline and his collaborators (c.f. Harvey Lectures 1941–42), but even though many histological investigations have been carried out in the past (the best of which is Watase's, Johns Hopkins Univ. Cir. Vol. VIII, No. 70, 1888–1889), the anatomy of this eye has remained obscure. For this reason Dr. H. G. Wagner and I have been investigating the histology further using modern techniques. We have benefited from Dr. D. Bodian's advice and would like to thank Messrs. E. F. MacNichol Jr., J. H. Mendelson, and M. L. Wolbarsht for their help.

The lateral eyes of *Limulus* are compound eyes and each contains about 600 ommatidia. Every ommatidium is composed of a lenticular portion of transparent chitin and a sensory portion containing two cell types, the retinula and the eccentric cells. In each ommatidium there are about a dozen retinula cells approximately 130 micra long and 30 micra thick arranged radially and giving rise to axons from their proximal ends. On the axial border of each of these cells is a highly refractile structure, the rhabdomere, which is characteristic

of a visual sense cell and has its counterpart in the outer segment of the rod and cone of the vertebrate retina. Located proximally and eccentrically in each ommatidium is one eccentric cell 40 by 50 micra which not only gives rise to an axon that travels proximally, but also to a short process that goes distally, down the axis of the ommatidium in a space surrounded by the rhabdomeres. The axons leave the proximal portions of the ommatidia and enter a network of nerve fibers, called the "plexus," in which the course of the individual fibers is difficult to trace. They emerge from this "plexus" to join in bundles and form the optic nerve. The axons of the eccentric cells, which are about twice the diameter of the axons of the retinula cells, appear to form interconnections with one another in the "plexus." These supposed interconnections are of importance because they may provide an explanation for the observation made by Dr. Hartline (c.f. Federation Proceedings, Vol. 8, No. 1, March 1949) that the activity of visual receptors in one area of the eye is inhibited by illuminating other areas of the eye that may be as much as several millimeters away.

The optic nerve is composed of several large nerve bundles containing a total of roughly 5000 nerve fibers, so there are almost as many nerve fibers as there are cells in the sensory portion of the eye. These nerve fibers vary in diameter from 1 to 12 micra with the principle mode of the distribution at 2.5 micra. A study is in progress to find out if there is a secondary mode that is statistically significant, as might be expected if the size difference of the axons of the retinula and eccentric cells is preserved in the optic nerve.

Dr. Hendrix: Mr. Miller's paper will be discussed by Dr. Hartline.

Dr. Hartline: Mr. Miller's study illustrates both the advantages and disadvantages of the comparative approach in the study of fundamental problems in biology. It is often desirable to go rather far afield in the animal kingdom in the search for a form of life in which the fundamental mechanism that one is studying can be observed in rather pure form. It was this that led us to study the eye of *Limulus* where the primary photoreceptor elements were readily available and where the very great complexity of nervous organization such as is observed in the vertebate retina could be avoided. The advantages of this approach have been great, for this preparation has afforded us an opportunity to study the relatively simple sensory activity of single visual receptor elements. In addition we have found a relatively simple example of physiological interaction between the elements of the eye in the form of mutual inhibitory influences. Since the inhibitory effect that a sensory element exerts upon its neighbors is greater the stronger the illumination upon that element, it is clear that in the eye as a whole elements that are brightly illuminated will inhibit the activity of elements in the more dimly lighted regions more strongly than will the dimly illuminated elements inhibit the activity from the brightly lighted areas. Thus, contrast is enhanced. Here we have a simple mechanism for an important visual phenomenon. However, when we attempt to determine the anatomical basis for this physiological observation, we are at a disadvantage, for the histological techniques worked out for vertebrate tissues do not give satisfactory results when applied to this invertebrate. Mr. Miller has had to find his own way through these difficulties and the progress that he has made in a relatively short time has been admirable. However, we are still far from a complete understanding of the neural connections in this eye and we hope that Mr. Miller will be sufficiently encouraged by what he has already obtained to continue this work.

Dr. Hendrix: Is there any further discussion or are there any questions on Mr. Miller's paper? The third paper this evening will be given by Mr. George W. Settle.

LOCALIZATION OF THE HEMOGLOBIN FORMING AREAS IN THE CHICK EMBRYO

For the past thirty years much of the attention of the experimental embryologist has been directed to determining what cells and areas of the undifferentiated embryo will later give

rise to specific tissues and organs. In this period most of the potential tissue forming areas of the chick embryo have been mapped with some care, but only a limited amount of work has been done on the potential hemoglobin-forming areas. In this investigation we have determined the location of these areas in each of ten stages of development, ranging from the earliest embryos available, at zero hours of incubation, to approximately seven somites, when hemoglobin is actually forming.

Technically this involved incubating fertile eggs for a suitable period, removing the embryo, sketching it with a camera lucida, cutting it into pieces with fine steel needles, transferring these pieces to tissue culture plates, and then watching them to see which would form hemoglobin. Twenty-five hundred cultures from 490 embryos were studied in this fashion. The results from a large number of cases at each stage were summated, first determining the boundaries in the vertical and transverse planes by vertical and transverse incisions at different levels, next determining the anterior limit of the area in the mid-line by a modified v-cut, and finally trimming these straight boundaries by a variety of curved cuts.

A study of the maps thus constructed shows the areas to occupy the posterior half of the *area pellucida* in the pre-streak blastoderm; to move medially and caudad, presumably invaginating through the primitive streak, and then to expand peripherally in the mesoblast of the surrounding *area opaqua* in a broad u-shaped pattern to form the *area vasculosa*. These changes in the location and configuration of the potential areas are generally compatible with the cell movements which are known to occur in the chick. These include the medial and caudal migration of the epiblast, its invagination, the peripheral expansion of the mesoderm, the relative backward growth of the embryo proper upon the *area opaqua*, and the regression of the primitive streak.

This compatibility tends, on one hand, to confirm the existence of these cell movements, and on the other hand, to suggest that the potential hemoglobin-forming areas are determined at a very early period, and later migrate to the site of their final differentiation.

Dr. Hendrix: Mr. Settle's paper will be discussed by Dr. Corner.

Dr. Corner: At the Carnegie Embryological Laboratory, where Mr. Settle has been working, we cannot take any particular credit for the investigation which he has reported this evening. He brought the problem with him when he came here as a medical student from Homewood. We gave him a corner in the laboratory and, outside of furnishing him with a table and our good wishes and buying these four hundred-odd eggs for him, we had almost nothing to do with what he has accomplished. He has had frequent conversations with us, telling us what he has told you tonight, but there has been no call for our advice or leadership. In his treatment of the subject he gave a clear statement of what this is all about and I would like only to say a word about the importance of the third point of his conclusions to an audience of this kind.

At the Carnegie laboratory we receive frequent visits from pediatricians and other clinicians who are interested in congenital deformities and anomalies. Thinking that such changes to the embryo may be due to something which happens to the mother during gestation, as has been discovered in regard to german measles. They are interested in looking back over the history of the pregnancy in order to link the anomaly with some specific illness or other event. The reasoning involves applying our knowledge of embryology by noting the relation between the time of the injurious condition and the state of development at that time. If for example there is a deformity of the arm, we look to see if the history of the mother gives anything to account for the injury at the time when the arm was developing. Mr. Settle's contribution, however, emphasizes the fact that the formation of a given part of the body may have begun before you can see that part at all. Before the arm bud is visible, the cells are already differentiated which will form that arm. In like manner, the tissues that form red cells and hemoglobin are marked out before there is any hemoglobin in the

body at all. Therefore it is going to take more knowledge of the invisible precursory differentiation of various regions of the body before we have clear clues to the exact time at which damage by infection or other disturbance of the mother might cause particular injuries of the embryo.

This is merely one of the important points waiting to be elucidated by studies like this on chicks, whereas the hope of doing such tests on mammalian embryos in an extensive way is still in the future.

Dr. Hendrix: Are there any further questions or discussion on Mr. Settle's paper? The fourth paper this evening will be given by Mr. Henry N. Wagner, Jr.

EXPERIMENTAL AND CLINICAL RESULTS WITH INTRAMUSCULAR TERRAMYCIN

Parenteral therapy is of potential value in the treatment of infectious diseases in patients who are unconscious or who suffer from nausea, vomiting, diarrhea, or diseases characterized by poor absorption from the gastro-intestinal tract. At present there is no broad-spectrum antibiotic that can be given intramuscularly. When terramycin hydrochloride was combined with sodium glycinate and procaine hydrochloride and injected into dogs, they showed no evidence of pain, and no histological evidence of local necrosis if the concentration of terramycin was below 33 milligrams per cc. of vehicle. Extensive observations for local toxicity were made in twenty adult male patients who were treated with intramuscular terramycin while hospitalized. The minimum number of injections that each patient received was 24. Six received over 75 injections at 6 hour intervals. One patient received over 110 injections. The dose was 100 mg. of terramycin in 3 cc. of vehicle injected into the gluteal muscles. No patient gave evidence of significant pain at the site of injection. There were no skin lesions, local abscesses, heat, or erythematous reactions. No systemic side reactions were observed.

The first therapeutic trial of the drug was a study of its effectiveness in the treatment of experimental cholangitis produced in 32 dogs by the injection of cultures of *E. coli* and *Strep. faecalis* into the biliary tract after complete ligation of the common bile duct. Intramuscular therapy modified favorably the course of this experimental infection. The results obtained in the terramycin treated dogs compare favorably with those obtained in dogs treated with combined penicillin and dihydrostreptomycin.

To evaluate the preparation under controlled clinical conditions, 36 out-patients with chronic urinary tract infections were selected for intramuscular terramycin therapy. Before therapy, all patients were examined twice weekly for a minimum of two weeks. All showed persistent infection. The most commonly used dosage schedule was 100 mg. of terramycin in 3 cc. of buffered vehicle every 12 hours for a total of 14 doses. Blood and urine levels were determined on several patients. After completion of therapy, follow-up examinations were made at 24 hours, 7 days, and one month. The intramuscular terramycin therapy was of value in the therapy of at least 30% of chronic refractory urinary tract infections, especially when caused by the coli-aerogenes group of organisms. Appearance of new organisms—*Proteus*, *Strep. faecalis*, and *Staph. aureus*—prevented the cure of many patients.

Dr. Hendrix: The discussion of Mr. Wagner's paper will be by Dr. Mirick.

Dr. Mirick: When Mr. Wagner came to see me at the end of last summer with his general program in mind we were confronted with several problems. The first was what kind of patients to select for a study like this, and the second was how to set up the study in such a way that the results would have some meaning. After considerable conversation we decided to pick this type of patient with chronic urinary tract infection for several reasons. First, there are quite a number of them. Secondly, these patients were likely to be grouped in cer-

tain localities of the hospital where it would be possible to see them without too much searching about. Thirdly, the patients studied were resistant to other forms of treatment so that any improvement might reasonably be credited to therapy rather than to a natural remission of the disease.

At a meeting of the Therapeutic Trials Committee it was originally planned that alternate cases be treated immediately and alternate cases have treatment withheld for two weeks. However, Mr. Wagner found it possible to withhold treatment on all patients for at least two weeks, thus making each patient his own control and allowing more patients to be included in the study. I think that the results he has shown indicate that it is possible with a small number of patients to reach conclusions which seem to have meaning, if the study is properly set up.

I believe he has demonstrated the effectiveness of this drug in the doses employed, which are relatively small, to cure at least a small proportion of patients with chronic urinary tract infection who have been refractory to all other forms of treatment that may have been employed up to the time of this study. Moreover, I think that it is a very worthwhile contribution to be able to demonstrate that there is now a preparation of this antibiotic which can be used effectively and without toxicity by the intramuscular route. It seems clear that such a preparation will not find its greatest usefulness in treating more patients of this same type who probably could be treated more easily and more effectively with larger doses by mouth. It would have usefulness in treating patients in the hospital who were unable to take oral therapy for a variety of reasons.

Dr. Hendrix: Dr. Zubrod.

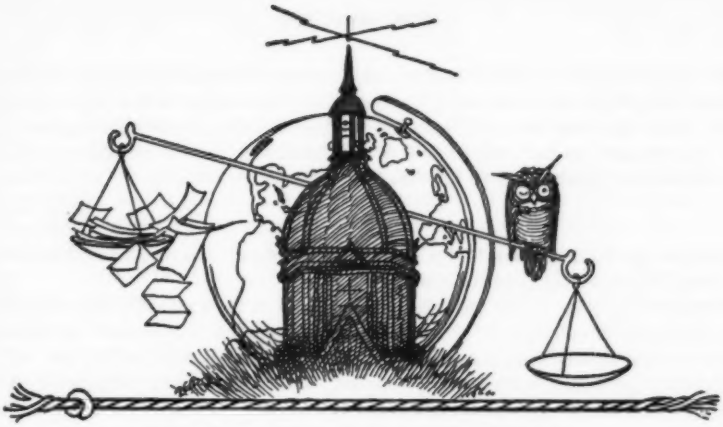
Dr. Zubrod: Mr. Wagner has certainly demonstrated that it is possible to cure some types of infections by the intramuscular administration of one of the broad-spectrum antibiotics, namely, terramycin. I think it is important for this audience to realize that there are some limitations in the use of this particular product. As far as I know, it is not commercially available. It has a certain amount of instability. When terramycin is mixed with sodium glycinate it is perfectly stable for a number of days. The preparation used here has in addition to terramycin and glycinate buffer, a small amount of procaine. For some reason, in this mixture, terramycin is quite unstable either *in vitro* or after injection into infected mice, so that in 30 minutes after putting it into solution, there is a considerable reduction in antibacterial potency. Mr. Wagner has avoided this loss of activity by making up all the solutions and injecting them immediately. Mr. Wagner is to be congratulated on the way in which he has been able to make a useful study of such complex problems. I think he is in a fine position to make in future years an overall study of comparative effectiveness of the various and varied products which are available for the treatment of urinary tract infection.

Dr. Hendrix: Are there any further questions or discussion? Dr. Fisher.

Dr. Fisher: It would be interesting to know in this sort of study how many of the organisms become resistant. Mr. Wagner says that *E. coli* is pretty well knocked out, but some of the strains persisted. I wonder if these strains and the other bacteria which appeared became resistant to terramycin.

Mr. Wagner: Dr. Fisher, we did paper disk sensitivity tests on practically every organism that was present, but we have never correlated these data with the other factors. In most cases, the organisms that persisted were very resistant to terramycin.

Dr. Hendrix: If there is no other discussion at this time, the meeting is adjourned.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Doctors in Blue: The Medical History of the Union Army in the Civil War. By GEORGE WORTHINGTON ADAMS. 253 pp., $4.00. *Henry Schuman, Inc., New York.*

The first principles of modern military medicine were learned in the two major wars of the 1850's to 1860's, the Crimean and American Civil Wars. It is fitting, therefore, to have such a thorough review of "The Medical History of the Union Army in the Civil War" as *Doctors in Blue.*

At Bull Run, the first major battle of war, the Army discovered that it had much to learn both militarily and medically. The prime movers in reforming the Medical Department were the Sanitary Commission, Surgeons-General Hammond and Barnes, and Jonathan Letterman. It was principally through their efforts that, considering the primitive state of medicine and surgery, the medical and sanitary record of the Civil War was on the whole good.

No one can really be aware of the medical advances in the past hundred years until he reads the description in the chapters on "Wartime Surgery" and "Operations and Infections." The modern medical student will undoubtedly be appalled by the descriptions of amputations performed with bloody clothes, hands, instruments, used sponges, and dressings until he realizes that in those days "laudable pus" was still expected and looked for hopefully.

Sanitation, of course, was more important, for it was not until the World Wars of the 20th Century that enemy action caused more casualties than disease. The most prevalent military disease now known as "The GI's" was then called "The Tennessee Quickstep." The Civil War was the last major conflict in which advances in sanitation and hygiene were greater than those in medicine and surgery.

The major medical contributions were administrative advances. Doctors did not receive full military rank until the Civil War. The modern chain of evacuation and the field hospital first appeared at this time. The Army Nurse Corps had its inception during the war after it was discovered that women appointed by a civilian who were past thirty and "plain almost to repulsion in dress, and devoid of personal attractions" did not always make good nurses.

The subject matter of *Doctors in Blue* makes the book fascinating reading in spite of its author's rather dull, flat style, and primitive medical knowledge. It is a pity, especially in view of these flaws that Adams' original doctoral dissertation, "Somewhat longer and profusely documented version" could not have been published. As it is, however, it will probably continue for sometime to be the definitive work on the subject.

G. W. C. Jr.

Grundlagen der Strahlentherapie. By Richard Kurt Kepp. 356 pp., Ganzleinen DM 38. *Georg Thieme Verlag, Stuttgart, Germany.*

Although the author of this book, a recent addition to the publisher's impressive list of works dealing with radiology, is Professor of Obstetrics and Gynecology at Gottingen, perusal of the text at once dispels any doubts of his ability to deal with radiation.

The text reads with the fluency and continuity of a spontaneously composed work by a single author. We note a gratifying constancy of style and orientation, and there are none of the difficulties one so often encounters in reading a work by several authors or one composed by the "clip and paste" method.

The first section deals in a very thorough way with the physics of radiation—discussing in sequence X-radiation, natural radioactivity, artificial radioactivity and radiation with accelerated particles. The author's background is such that the clinical application of the physical principles described are kept in the foreground.

The second section considers the biologic effects of radiation and progresses logically from molecular, bacterial and single cell alterations to changes caused in tissues and individual organs. There follows a good treatment of radiation effects on reproduction and offspring, and then a section devoted to the reaction of the organism as a whole to radiation. The body of the text concludes with a short section on protection and a rather extensive general discussion of radiation in the treatment of malignant disease. Mathematics is kept at a minimum. The illustrations are not numerous.

Space does not permit a detailed description of the features of this work, but in an attempt to summarize, one is impressed by the facility with which theoretical and clinical principles are interdigitated. One obtains a well rounded point of view and a good perspective of radiology from this logical, unhurried and well integrated treatise. An English translation might well be favorably received.

D. J. T.

Therapeutic Studies on Psychotics. By Julius I. Steinfeld. 262 pp. *Forest Press, Publishers, Des Plaines, Ill.*

The bright red dust jacket of this book is no less startling than its contents. It contains four studies of treatment of psychotics which, as the writer says "constitute a unitary expression of a therapeutic enthusiasm born out of despair with the inadequacy of isolated and exclusive approaches." The first chapter, comprising about half the book, describes 18 schizophrenics successfully treated with psychotherapy, and one failure. It emphasizes the necessity for genuine and flexible emotional interplay between therapist and patient. The therapist tried to fit his behavior to the needs of the particular case, being strict with some, permissive with others, enlisting the aid of a second therapist at times, manipulating the environment extensively, and even encouraging a homosexual relationship between two of his patients. Throughout the author wisely stresses the importance of the therapist's dedication to his patients' welfare, and has the courage to state that his own gratification at a successful outcome is an important factor in sustaining his efforts through the storms of therapy.

The second chapter presents a clinical and statistical review of 500 patients treated with

various forms of shock therapy with results which are considerably better than those usually reported. The same willingness to try anything is in evidence—various positions of electrodes, various strengths and durations of current, multiple convulsions (as many as four in 20 minutes) and maintenance shock treatment over years.

The third chapter presents evidence that testosterone helps to stabilize patients whose emotional upsets are temporally related to their menses, and the last adduces some experimental support for the hypothesis that the effectiveness of shock treatment is linked to the extent of the shift in acid-base balance it produces.

The book is interestingly written and evinces a genuinely psychobiological viewpoint although it is somewhat topheavy with psychoanalytical speculation. The writer makes the impression of being an intuitive, resourceful, courageous person. The material, however, is woefully weak from the standpoint of scientific validity. Only successful cases are reported in detail. Many patients received several types of treatment simultaneously and successively, so that it is hard to disentangle just what might have accounted for their improvement, a concept which itself is admittedly not adequately defined. The possibility of natural fluctuations in the patients' condition is nowhere considered. The writer periodically stresses the tentativeness of his conclusions, but presents such an array of favorable results with so much enthusiasm that the reader is apt to forget that he has proved nothing. Experienced psychiatrists will find this book highly stimulating but it will dazzle and bemuse the uninitiated.

J. D. F.

Liver Injury: Transactions of the Ninth Conference April 27–28, 1950, New York. Edited by F. W. HOFFBAUER. 232 pp., $3.00. *Josiah Macy, Jr. Foundation, New York.*

This volume is a record of the Ninth Conference on Liver Injury, sponsored by the Josiah Macy, Jr., Foundation. As in previous years, the conference dealt with a number of widely different subjects. Perhaps the most exciting discussion was that of Kwashiorkor, by Dr. J. N. P. Davies, of Makere College Medical School of Uganda, East Africa.

It might not be out of place to make a few general comments about these published Transactions. The volumes are divided into a number of sections, each of which deals with a separate topic. In each section, one or more participants present, in a relatively formal manner, the results of their original researches. The discussion which ensues is recorded.

To what extent are these published Transactions useful? There is no question about the value of the formal papers. These are usually clear presentations of the current status of the investigative work of persons eminent in their respective fields. The style is generally lucid, since it is not confined to the rather strangulating forms of modern scientific writing. For these papers alone, the publication of the Transactions is a singular public service.

On the other hand, the record of the informal discussions which follow each paper is considerably less useful. Its entertainment value is undoubted. Even in the edited form in which they appear, the discussions bring out the personalities of the participants, and make the Transactions good bed-time reading. The reader wants to join in the conversation, pro or con. But the permanent value of these dialogues is open to question. Frequently the participants wander far from the topic. The absence of an index to each volume makes it impossible to refer to a particular bit of the discussion without considerable searching. The Transactions, then, are entertaining volumes, but some revision in the manner of presentation might increase their utility.

O. D. R.

Klinik und Therapie der Vergiftungen: By SVEN MOESCHLIN. 430 pp., Ganzleinen DM 45. *Georg Thieme Verlag, Stuttgart, Germany.*

The author of this volume has done a fine piece of work in incorporating within it the important clinical features and therapy of a large number of the commoner types of poisoning.

The subject is introduced with a section in which the frequency and distribution of poisonings, the general methods of treatment and the important therapeutic agents are considered. The remainder of the work is divided into five sections on inorganic poisons, organic poisons, plant poisons and their derivatives, food posions and animal poisons respectively. It is well illustrated with fifty-three figures.

This book has been written for the practicing physician who may encounter any kind of poisoning at any time. It gives him the important information about the occurrence, mechanism, pathology, symptoms, diagnosis, differential diagnosis and treatment of poisonings. In many instances illustrative case histories are given. For each poison, selected references are listed at the end of the discussion.

The rational therapeutic measures indicated are the best current practice; however, the author is to be criticized for his tendency to list, in addition, various so-called stimulants which frequently are of little value and if used indiscriminately may be deleterious. In cases where chemotherapeutic measures are indicated he consistently advises depot penicillin. Other preparations might be equally efficacious.

This book should be valuable to hospital libraries as a reference, and if translated, would be excellent for the practicing physician as there appears to be no book in English which is quite so complete and concise a source of guidance for the management of cases of poisoning.

E. H. D.

Prescription for Medical Writing. A Useful Guide to Principles and Practice of Effective Scientific Writing and Illustration. By EDWIN P. JORDAN and WILLARD C. SHEPARD. 112 pp., $2.50. *W. B. Saunders Co., Philadelphia, Pa.*

Voltaire is quoted as having apologized in a letter to a friend with the statement that he had not had time to make the letter brief. Certainly an incalculable amount of other people's time would be saved, if authors would take the necessary time to make their papers brief and to the point. To all those who desire to do so this book will be a real help.

The book contains wise advice on elementary statistics and on charts and diagrams. The chapter on illustrations is authoritative. It is written by an excellent medical artist, who has helped Saunders Company make a really worth-while contribution to the reproduction of medical illustrations.

The writing itself is brief and clear. An estimate of what is involved in writing an article is the fact that Chapter II is entitled "The First Draft" and Chapter V, "The Third Revision."

We can but hope that many people will study this opus, and then all our journals will be thinner. Hallelujah!

F. B. B.

Pharmacopoea Internationalis, 1st edition. Vol. 1. 406 pp., $5.00. Available in English, French and Spanish editions. *World Health Organization, Palais des Nations, Geneva.*

The purpose of a pharmacopeia is to compile standards of purity, potency and nomenclature for drugs which are in common use. Thus when a physician in this country prescribes a drug and writes after it U.S.P., he can be sure that the patient will receive a product with the characteristics listed in the U. S. Pharmacopeia. Other countries have different nomenclature, different standards and even different drugs, and pharmacists and physicians have long felt the need for international unification. Efforts in this direction have been underway

since 1874 and various working agreements have been made. The WHO has succeeded in publishing the first international pharmacopeia and the members of the commission are to be congratulated upon the result. It is a work of great simplicity and common sense and must surely succeed in its purpose.

The individual monographs are concise; common technical details are relegated to the appendices. Most of the truly useful drugs are included, although antibiotics and other recent developments are to be presented in Vol. 2. The material in each monograph is of course of interest only to the pharmacist; the physician would find helpful only a single table in the appendix where there is a list of the usual and maximal doses, either for a single or total daily dosage, of all the drugs in the pharmacopeia. Although the physician will rarely if ever have a need to consult this volume, he can at least applaud the ability of these internationally-minded pharmacists to succeed in an arena where statesmen have failed, and to compromise in a sensible and useful way.

C. G. Z.

Autopsy Diagnosis and Technic, 3rd edition. By OTTO SAPHIR. 471 pp., $6.00. *Paul B. Hoeber, Inc., New York.*

The instructions for performing a post-mortem examination are excellent and the descriptions of the gross appearance of tissues are good. A book to teach the interpretation of autopsy findings might include more material both clinical and morphologic but of course no textbook can replace experience in this regard. This book apparently is intended as a guide for the beginner in pathology or for the physician who does only an occasional autopsy, and for such indivuduals it could be very useful.

G. E. McK.

BOOKS RECEIVED FOR REVIEW

Biochemistry for Medical Students, 5th edition. By WILLIAM VEALE THORPE. 528 pp., $6.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Cold Injury. Transactions of the First Conference, June 4–5, 1951. Edited by M. IRENE FERRER. 248 pp., $3.25. *Josiah Macy, Jr. Foundation, New York.*

Cybernetics. Transactions of the Eighth Conference, March 15–16, 1951. Edited by HEINZ VON FOERSTER. 240 pp., $4.00. *Josiah Macy, Jr. Foundation, New York.*

Dynamic Psychiatry. Edited by FRANZ ALEXANDER and HELEN ROSS. 578 pp., $10.00. *University of Chicago Press, Chicago, Illinois.*

Elementary Medical Statistics. The Principles of Quantitative Medicine. By DONALD MAINLAND. 327 pp., $5.00. *W. B. Saunders Co., Philadelphia, Pa.*

The Fight against Tuberculosis. An Autobiography. By FRANCIS MARION POTTENGER. 276 pp., $4.00. *Henry Schuman, Inc. New York.*

Klinische Probleme der Vegetativen Regulation und der Neuralpathologie. By FERDINAND HOFF. 43 pp., Kart. DM 2.70. *Georg Thieme Verlag, Stuttgart, Germany.*

The Metabolic Response to Surgery (American Lecture Series). By FRANCIS D. MOORE and MARGARET R. BALL. 156 pp., $7.50. *Charles C. Thomas, Springfield, Illinois.*

The Rh Blood Groups and their Clinical Effects. Medical Research Council Memorandum No. 27. By P. L. MOLLISON, A. E. MOURANT and R. R. RACE. 72 pp., 3s. *Her Majesty's Stationery Office, London, England.*

Surgery and the Endocrine System. By JAMES D. HARDY. 153 pp., $5.00. *W. B. Saunders Co., Philadelphia, Pa.*

Surgical Technique. By STEPHEN POWER. 380 pp., $6.50. *J. B. Lippincott Co., Philadelphia, Pa.*